28 Rec'd 21 JAN1998 09/000004

FORM PTO-1390 (REV 10-94)	U S DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE	ATTORNEY'S DOCKET NUMBER
TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371		**600.314USWO**
		U S APPLICATION NO (If known, see 37 C F R 1 5)

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/US96/12067	19 July 1996 (19.07.96)	21 July 1995 (21.07.95)

TITLE OF INVENTION
ANALYSIS OF ALPHA INTEGRINS FOR THE DIAGNOSIS OF DIABETIC NEPHROPATHY

APPLICANT(S) FOR DO/EO/US
TSILIBARY, Photini-Effie; CHARONIS, Aristidis, S.; SETTY, Suman; and MAUER, Michael

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. X This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. [ ] This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. [ ] This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(l).
4. [ ] A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. X A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. [ ] is transmitted herewith (required only if not transmitted by the International Bureau).
   b. has been transmitted by the International Bureau.
   c. ☒ is not required, as the application was filed in the United States Receiving Office (RO/US)
6. [ ] A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. [X] Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. [ ] are transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ have been transmitted by the International Bureau.
   c. [ ] have not been made; however, the time limit for making such amendments has NOT expired.
   d. [X] have not been made and will not be made.
8. [ ] A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. [ ] An oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)).
10. [ ] A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern document(s) or information included:

11. [ ] An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. [ ] An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. [ ] A FIRST preliminary amendment.
    [ ] A SECOND of SUBSEQUENT preliminary amendment.
14. [ ] A substitute specification.
15. [ ] A change of power of attorney and/or address letter.
16. [ ] Other items or information:

U.S. APPLICATION NO (If known, see 37 C F R 1 5)X	INTERNATIONAL APPLICATION NO	ATTORNEY'S DOCKET NUMBER
	PCT/US96/12067	600.314USWO

17. x The following fees are submitted:

CALCULATIONS PTO USE ONLY

BASIC NATIONAL FEE (37 CFR 1.492(a) (1)-(5)):

Search Report has been prepared by the EPO or JPO **$930.00**

International preliminary examination fee paid to USPTO (37 CFR 1.482) **$720.00**

No international preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2)) **$790.00**

Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO **$1070.00**

International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4) **$98.00**

ENTER APPROPRIATE BASIC FEE AMOUNT =				**$1070.00**	
Surcharge of **$130.00** for furnishing the oath or declaration later than [ ] 20 [ ] 30 months from the earliest claimed priority date (37 CFR 1.492(e)).				**$**	
CLAIMS	NUMBER FILED		NUMBER EXTRA	RATE	
Total claims	30	-20 =	10	X **$22.00**	**$ 220.00**
Independent claims	4	-3 =	1	X **$82.00**	**$ 82.00**
MULTIPLE DEPENDENT CLAIM(S) (if applicable)				+ **$250.00**	**$**
TOTAL OF ABOVE CALCULATIONS =					**$1372.00**
Reduction by 1/2 for filing by small entity, if applicable. Verified Small Entity Statement must also be filed (Note 37 CFR 1.9, 1.27, 1.28).					**$**
SUBTOTAL =					**$1372.00**
Processing fee of **$130.00** for furnishing the English translation later than [ ] 20 [ ] 30 months from the earliest claimed priority date (37 CFR 1.492(f).				+	**$**
TOTAL NATIONAL FEE =					**$1372.00**
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). $40.00 per property				+	**$**
TOTAL FEES ENCLOSED =					**$1372.00**
				Amount to be: refunded	**$**
				charged	**$**

a. X A check in the amount of ~~$1284.00~~ $1372.00 to cover the above fees is enclosed.

b. [ ] Please charge my Deposit Account No. _____ in the amount of $ _____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. X The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 13-2725. A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SIGNATURE:

Steven C. Bruess
NAME

34,130
REGISTRATION NUMBER

SEND ALL CORRESPONDENCE TO:
Denise M. Kettelberger
MERCHANT, GOULD, SMITH, EDELL, WELTER & SCHMIDT, P.A.
3100 Norwest Center
90 South Seventh Street
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Minneapolis, Minnesota 55402

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	TSILIBARY, ET AL.	Examiner:	UNKNOWN
Serial No.:	09/000,004	Group Art Unit:	UNKNOWN
Filed:	JANUARY 21, 1998	Docket No.:	600.314USWO
Title:	ANALYSIS OF ALPHA INTEGRINS FOR THE DIAGNOSIS OF DIABETIC NEPHROPATHY		

CERTIFICATE UNDER 37 CFR 1.8: The undersigned hereby certifies that this correspondence is being deposited in the United States Postal Service, as first class mail, with sufficient postage, in an envelope addressed to: BOX PCT, Commissioner for Patents, Washington, D.C. 20231 on May 23, 2001.

By: Anne Harrison
Name: Anne Harrison

PRELIMINARY AMENDMENT

BOX PCT
Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

IN THE ABSTRACT

Please insert the attached Abstract into the specification of the application as the last page thereof.

IN THE SPECIFICATION

Page 1, after the title, please insert the following paragraph:

--This application is a Nonprovisional of U.S. Provisional Application No. 60/001,387 filed on July 21, 1995; U.S. Provisional Application No. 60/001,861 filed on August 3, 1995; and U.S. Provisional Application No. 60/016,700 filed May 2, 1996.--

IN THE CLAIMS

Please cancel claim 1-30 and enter new claims 31-56.

31. (NEW) A method for identifying a mammal having or at risk for developing glomerulonephropathy comprising the steps of:

analyzing integrin subunit expression in a mammalian tissue sample known to contain cells expressing α1 and α2 integrin subunits and in a control tissue sample; and

correlating a decreased level of α1 integrin subunit expression or an increased level of α2 integrin subunit expression in the sample tissue as compared with the control tissue with the presence of or risk of developing nephropathy.

32. (NEW) A method for identifying a mammal having or at risk for developing glomerulonephropathy comprising the steps of:

analyzing integrin subunit expression in a mammalian tissue sample known to contain cells expressing α1 and α2 integrin subunits and in a control tissue sample; and

correlating a decreased level of α1 integrin subunit expression and an increased level of α2 integrin subunit expression in the sample tissue as compared with the control tissue with the presence of or risk of developing nephropathy.

33. (NEW) The method of claim 31, wherein the mammal is a human.

34. (NEW) The method of claim 31, wherein the tissue sample is a kidney biopsy, a skin biopsy, or blood.

35. (NEW) The method of claim 31, wherein said analyzing comprises *in situ* hybridization.

36. (NEW) The method of claim 35, wherein said *in situ* hybridization comprises PCR enhanced *in situ* hybridization.

37. (NEW) The method of claim 31, wherein said analyzing comprises isolating RNA from the sample.

38. (NEW) The method of claim 31, wherein said analyzing comprises PCR amplification of alpha integrin subunits, and comparison of the relative amounts of $\alpha 1$ and $\alpha 2$ integrin subunits amplified in the sample and in the control.

39. (NEW) The method of claim 38, wherein the integrin subunits are analyzed with a nucleic acid probe comprising 15 or more consecutive nucleotides of $\alpha 1$ integrin nucleotides 1-3900 (SEQ ID NO: 1).

40. (NEW) The method of claim 38, wherein the integrin subunits are analyzed with a nucleic acid probe comprising 15 or more consecutive nucleotides of $\alpha 2$ integrin nucleotides 1-1800 (SEQ ID NO: 3).

41. (NEW) The method of claim 39, wherein said nucleic acid probe comprises 15 or more consecutive nucleotides of $\alpha 1$ integrin nucleotides 267-645, 1530-1990 or 2278-2728 (SEQ ID NO: 1).

42. (NEW) The method of claim 40, wherein said nucleic acid probe comprises 15 or more consecutive nucleotides of $\alpha 2$ integrin nucleotides 320-800, 452-893, or 1607-1732 (SEQ ID NO: 3).

43. (NEW) The method of claim 31, wherein said analyzing comprises incubating the sample with an anti-integrin subunit antibody.

44. (NEW) The method of claim 31, wherein the control sample is from a mammal having no history of hypertension.

45. (NEW) The method of claim 31, wherein an increase of about 25% - 100% in the level of $\alpha 2$ integrin subunit expression in the sample tissue as compared with the control is correlated with nephropathy.

46. (NEW) The method of claim 31, wherein a decrease of about 25% - 100% in the level of α1 integrin subunit expression in the sample tissue as compared with the control is correlated with nephropathy.

47. (NEW) A method for identifying a mammal having diabetes who has or is at risk for developing secondary pathological changes associated with diabetes comprising the steps of:

analyzing integrin subunit expression in a mammalian tissue sample known to contain cells expressing α1 and α2 integrin subunits and in a control tissue sample; and

correlating a decreased level of α1 integrin subunit expression and/or an increased level of α2 integrin subunit expression in the sample tissue as compared with the control tissue with the presence of or risk of developing secondary pathological changes associated with diabetes.

48. (NEW) A kit for the diagnosis of nephropathy comprising:

a set of hybridization probes or antibodies capable of detecting each of α1 and α2 integrin subunit expression in a tissue sample.

49. (NEW) The kit of claim 48, further comprising nucleic acid primer pairs for amplification of α1 and α2 integrin subunits.

50. (NEW) The kit of claim 48, comprising one or more of the following primers for amplification of α1: Sequence ID Nos. 5, 6, 7, 11, 12, and 13.

51. (NEW) The kit of claim 48, comprising one or more of the following primers for amplification of α2: comprise Sequence ID Nos. 8, 9, 10, 14, 15, and 16.

52. (NEW) The kit of claim 48, further comprising α1 and α2 integrin subunit standards.

53. (NEW) The kit of claim 48, wherein said hybridization probe comprises 15 or more consecutive nucleotides of α1 integrin nucleotides 1-3900 (SEQ ID NO: 1).

54. (NEW) The kit of claim 48, wherein said hybridization probe comprises 15 or more consecutive nucleotides of $\alpha 2$ integrin nucleotides 1-1800 (SEQ ID NO: 3).

55. (NEW) The kit of claim 53, wherein said hybridization probe comprises 15 or more consecutive $\alpha 1$ integrin nucleotides 267-645, 1530-1990, or 2278-2728 (SEQ ID NO: 1).

56. (NEW) The kit of claim 54, wherein said hybridization probe comprises 15 or more consecutive $\alpha 2$ integrin nucleotides 320-800, 452-893, or 1607-1732 (SEQ ID NO: 3).

REMARKS

Claims 1-30 have been canceled, and new claims 31-56 have been added. A statement to this effect is reiterated on a separate page under the heading of "Marked Up Claims." New claims 31-56 better conform to the format used in U.S. practice. No new matter has been added.

A new Abstract, conforming with that appearing on the publication page of the WIPO application, has been submitted on a separate page as required.

Respectfully submitted,

MERCHANT & GOULD P.C.
P.O. Box 2903
Minneapolis, Minnesota 55402-0903
(612) 332-5300

Date: May 23, 2001

Denise M. Kettelberger

Denise M. Kettelberger, Ph.D.
Reg. No. 33,924
DMK:KC:PSTpmc

Serial No.: 09/000,004

MARKED UP SPECIFICATION AND CLAIMS

In the specification:

Page 1, after the title, the following paragraph was inserted:

--This application is a Nonprovisional of U.S. Provisional Application No. 60/001,387 filed on July 21, 1995; U.S. Provisional Application No. 60/001,861 filed on August 3, 1995; and U.S. Provisional Application No. 60/016,700 filed May 2, 1996.--

In the claims:

Claims 1-30 have been cancelled.

Claims 31-56 have been added.

ABSTRACT

Analysis of alterations in integrin subunit expression, particularly $\alpha 1$ and/or $\alpha 2$ integrin subunit expression from integrin producing cells as compared to normal controls as a diagnostic method to identify individuals who have or are predisposed to pathologies associated with altered matrix deposition, such as diabetic renal nephropathy.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Tsilibary, Photini–Effie, et al.
Serial No: PCT/USUnknown (based on PCT/US96/12067) Int'l Filing Date: Concurrently Herewith
Docket No.: 600.314USWO Due Date: January 21, 1998
Title: ANALYSIS OF ALPHA INTEGRINS FOR THE DIAGNOSIS OF DIABETIC NEPHROPHATHY

CERTIFICATE UNDER 37 CFR 1.10:
"Express Mail" mailing label number: EM045419937US
Date of Deposit: January 21, 1998
I hereby certify that this paper or fee is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1.10 on the date indicated above and is addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231.

By: _____
Name: William Smith

Assistant Commissioner for Patents
Attn: BOX PCT
Washington, D.C. 20231

Sir:

We are transmitting herewith the attached:

☒ Transmittal sheet in duplicate containing Certificate under 37 CFR 1.10.
☒ A check in the amount of $ 1372.00 to cover filing fee.
☒ A return postcard.
☒ Other: PTO–1390 (2 pages)

Please charge any additional required fees or credit overpayment to Deposit Account No. 13–2725 A duplicate copy of this sheet is enclosed.

MERCHANT, GOULD, SMITH, EDELL, WELTER & SCHMIDT
3100 Norwest Center, Minneapolis, MN 55402 (612/332–5300)

By: _____
Name: Steven C. Bruess
Reg. No.: 34,130
Initials: SCB/IPD/KMC/mls

09/000004
PCT/US96/12067
10 Rec'd PCT/PTO 21 JAN 1998

ANALYSIS OF ALPHA INTEGRINS FOR THE DIAGNOSIS OF DIABETIC NEPHROPATHY

Background of the Invention

Diabetic nephropathy is a major cause of renal failure in the U.S. and develops in approximately 30% of insulin dependent diabetes mellitus (IDDM) patients. Recent studies by the Diabetes Control and Complications Trial Group have indicated that intensive insulin treatment substantially reduces the risk of developing complications, including nephropathy. However, the cost and effort of the intensive therapy, as well as the danger of hypoglycemic attacks dictate that this treatment should be limited to those patients who are prone to develop complications. It follows that an early selection of these diabetic subjects would be extremely helpful, but currently there are no adequate predictors available for clinical use.

Metabolic imbalance caused by hyperglycemia has been implicated as a major factor in the development of this condition and is associated with a genetic tendency to develop nephropathy. A prominent expansion of the mesangium with changes in the composition of the mesangial matrix have been observed in diabetic nephropathy (Williamson et al., *Diabetes Met. Rev.* 4:339 (1988), Steffes, M.W., et al. *Diabetes* 38:1077-81 (1989)).

Studies performed with human and experimental animal mesangial cells cultured in high-glucose medium have demonstrated an increased synthesis and accumulation of matrix proteins, namely collagens, including collagen type IV and fibronectin. This suggests that hyperglycemia plays a role in the mesangial changes of diabetic nephropathy. Ayo, S.H., et al. (1990a), *Am. J. Pathol.* 136:1339-1348; Nahman, N.S., et al., *Kidney Int.* 41:396-402 (1992); Danne, T., et al., *Diabetes* 42:170-177 (1993). The changes in the matrix secretion pattern of the cell are mediated either directly by hyperglycemia or by the glycation of mesangial matrix on prolonged exposure to high levels of glucose. Studies have demonstrated that cultured mesangial cells are influenced by the glycation of matrix leading to altered cell adhesion, spreading and proliferation. Since collagen IV (cIV) is the major component of the mesangial matrix (about 60%), changes in the interactions between this major mesangial glycoprotein and mesangial cells may play an important role in the pathology of diabetic nephropathy. Kim, Y., et al., *Am. J. Pathol.* 138:413-420 (1991). The changes in matrix deposition

are secondary in time to insulin insufficiency. Altered matrix deposition including basement membrane thickening is also found in a variety of arterioles and arteries in patients with diabetes mellitus. Altered matrix deposition is found in the pancreas of diabetic patients. Altered matrix deposition puts diabetic patients at risk for developing secondary pathological changes including, but not limited to nephropathy, myocardial infarction, cerebral stroke, problems associated with reduced circulation, retinopathy, neuropathies and the like.

Cell-matrix interactions are mediated, for the most part, by a family of receptors known as integrins. The very late antigen (VLA) subgroup of integrins which share a common β1 chain, include the cell membrane receptors for cIV, α1β1 and α2β1. Although integrins are mainly studied for their role in cell differentiation, migration and signaling events, they may also be involved in the maintenance of tissue structure. For instance, cells can modify their matrix by altering the production of matrix proteins and/or by regulating matrix organization. Cells cultured under high glucose conditions resulted in an increased production of matrix components by mesangial cells. (Kashgarian, M., et al., *Kidney Int.* 41:524-529 (1992).) The balance of cell surface integrin expression has been demonstrated to be altered in various disease states including inflammation and malignancy (Waes and Carey, *Otolarnyngologic Clinics of North America* 25(5):1117 (1992); Adams, J.C., et al., *Cell* 63:425-435 (1990); Rozzo et al., *FEBS Letters* 332:263 (1993)). This altered expression has been associated with altered adhesion to extracellular components.

Presently, the only earliest available indicator of kidney changes is microalbuminuria which occurs after the appearance of nephropathic changes. Yet only a percentage of individuals with microalbuminuria go on to develop glomerulopathy. Individuals at risk for developing glomerulopathy are best treated with intense glucose-modulating therapies that have their own risk. Often physicians are hesitant to place individuals with microalbuminuria on such therapies since the majority of these patients do not proceed to glomerulopathy. Biopsies indicating the accumulation of matrix accompanying the expansion of the mesangium occur at a point when the process has become irreversible. Therefore an early predictor of nephropathy or other disease states associated with altered matrix deposition would be beneficial as an indicator of those

patients who require stringent control of blood glucose levels to minimize nephropathic and other altered matrix deposition-associated disorders.

Thus, there is a need to identify markers associated with the changes seen in nephropathy and in other altered matrix deposition-associated disorders for the diagnosis of these disorders. There is a need to identify changes in regulation and function of integrins in diabetic patients and there is a need to develop a diagnostic test that can be used to identify patients who are likely to develop or have the early symptoms of nephropathy.

Summary of the Invention

Alterations in the amounts and patterns of alpha-integrin subunits has now been correlated to the onset of nephropathy. Analysis of alpha integrin subunit expression as compared with controls provides a diagnostic tool for the determination of patients likely to develop severe nephropathy and a method to monitor progress of disease during treatment protocols.

Cells that express alpha integrins, such as kidney tissue, fibroblasts, endothelial cells, and blood cells are analyzed for alpha integrin subunit expression, for example, by *in situ* hybridization methods. Changes in the amounts and pattern of integrin subunit expression as compared with control samples, is diagnostic of nephropathy and can be used to screen individuals, e.g., diabetic patients at risk for developing severe disease.

Analysis of $\alpha 1$, $\alpha 2$, $\alpha 3$, $\alpha 5$, and beta-1 integrin subunit expression as compared with control tissue expression is preferred. An increase in $\alpha 2$, $\alpha 3$, $\alpha 5$, or beta-1 integrin expression and/or a decrease in $\alpha 1$ expression is diagnostic of increased risk of nephropathy. An especially preferred diagnostic method is the comparison of $\alpha 1$ and $\alpha 2$ integrin subunit expression with control tissue. A pattern change including a decrease in $\alpha 1$ and an increase in $\alpha 2$ is diagnostic of increased risk of nephropathy or onset of the disease.

Brief Description of the Drawings

Figure 1 is a histogram summarizing results of *In situ* hybridization studies of rat control and diabetic tissue with $\alpha 1$ and $\alpha 2$ integrin probes.

Detailed Description of the Invention

Analysis of changes in the pattern of integrin subunit expression, particularly of alpha integrin subunits, is made by comparing expression in sample tissues as compared with tissue controls.

Tissue Samples:

The invention is directed to methods of detecting changes in α integrin subunit expression in cells, such as the cell populations (visceral epithelial, endothelial and mesangial and other matrix-producing cells) present in the glomerulus; and also in the tubules as well as including, but not limited to, fibroblasts (for example see D. Kyu Jin, et al. in *J. Am. Society of Nephrology*, 5(3): 966, 1994), epithelial, and endothelial cells from a variety of tissues and organs as well as blood cells including, but not limited to polymorphonuclear leukocytes, monocytes, and the like. Changes to blood cells, including leukocytes, have been reported in diabetic patients who develop nephropathy (Ng, et al. *Diabetologia* 33:278-284, 1990).

A change in the expression of α1 and α2 integrins has been detected in the studies disclosed here, under conditions of high glucose (i.e., about 25 mM) compared with low glucose (i.e., about 5 mM), in diabetic test animals *in vitro*, and in a human diabetic patient with neuropathy. Mesangial cells cultured in high glucose showed an increase in α2 integrin expression and a decrease in α1 integrin expression compared with mesangial cells grown under low glucose conditions. A change in expression of α integrins such as α1 and/or α2 subunits can be used to identify patients that have or will develop diabetic nephropathy. In view of these studies, it is believed that patients showing about a 25 to 100% decrease in α1 integrin and/or about a 25 to 100% increase in α2 integrin expression have a greater chance of developing diabetic nephropathy. The methods disclosed here are useful to identify diabetic patients at risk for developing diabetic nephropathy. The methods may also be useful to monitor progression of diabetic nephropathy. Patients identified as having a risk for developing or showing early symptoms of diabetic nephropathy can be placed on a strict glucose control regimen so that the development and/or progression of nephropathy can be inhibited.

Changes in integrin subunit expression in diabetic patients have been identified in cultured human skin fibroblasts taken from skin biopsies (D. Kyu Jin, et al., *J. Am.*

Soc. of Nephrology 5(3):966, 1994) suggesting that a variety of integrin-expressing cells could be monitored to identify individuals with a predisposition to nephropathy or to other complications associated with diabetes-induced altered matrix deposition.

Methods of Detecting a Change in Expression of α1 and/or α2 Integrin Subunits in Cells from Diabetic Patients

The methods of the invention are conducted with cell types that express alpha (α) integrin subunits. Preferably, to identify patients predisposed to nephropathy, the cells are obtained from tissue samples from biopsy of kidney tissue of diabetic patients. However, other cell types that express α integrin subunits can be utilized including, but not limited to, fibroblasts, endothelial cells, polymorphonuclear leukocytes, monocytes, and other blood cells. The amount of cells typically obtained is relatively small so that the detection methods selected are those that can detect and/or quantitate α integrin subunit expression in a small cell sample. These methods include, but are not limited to *in situ* hybridization, including polymerase chain reaction (PCR) enhanced *in situ* hybridization (also known as *in situ* PCR) and the like.

The cell samples are obtained from patients having diabetes but having no demonstrable symptoms or signs of nephropathy. The earliest change in nephropathy is the detection of microalbuminuria. Biopsy specimens may also be obtained from diabetic patients that may have early symptoms of nephropathy so that the progression of diabetic nephropathy can be monitored. Blood samples and skin biopsies also can be obtained from patients with diabetes and processed for either *in situ* hybridization or PCR enhanced *in situ* hybridization (also known as *in situ* PCR). Similarly, it is possible to perform *in situ* hybridization or PCR enhanced *in situ* hybridization using a cheek scraping or a scraping of other accessible tissue.

Biopsy tissue samples are usually about $1mm^3$ and are obtained using standard biopsy methods. Where the kidney is the organ selected for biopsy, kidney tissue from the cortical region is preferred although biopsy samples can be obtained elsewhere. Fibroblasts can be obtained from skin or any other tissue. The biopsy samples are then frozen in liquid nitrogen or fixed in 4% fresh paraformaldehyde and sectioned into 5 μm thick sections on silane-coated slides. The sections can then be treated with reagents to detect and/or quantitate α integrin expression in cells.

Blood cells and other α integrin expressing cells can also be analyzed for changes in α integrin subunit expression. These cells include fibroblasts, monocytes, polymorphonuclear leukocytes and other blood cells. Cells can be obtained and isolated from a blood or bone marrow sample. Methods for isolating particular cell types from a blood sample are well known in the art. Preferably leukocytes are isolated from blood by centrifugation, followed by hypotonic shock of residual blood cells as disclosed by Ng, et al. *Diabetologia* 33:278-284, 1990.

Rather than preparing cell sections, the sample of cells can be extracted to obtain nucleic acids using standard methods. The nucleic acids encoding α1 and/or α2 integrin subunits can be amplified using any of a variety of polymerase chain reaction methods. For example, changes in the level of expression of α1 and/or α2 integrins can be detected using a competitive PCR method as described by Gilland, G., *Proc. Natl. Acad. Sci. (USA)* 87:2725 (1990).

In a method of the invention, the level of α1 integrin expression is detected and/or quantitated in cells such as glomerular and tubular kidney cells. The level of α1 integrin expression can be detected using a variety of standard methods. The preferred methods are *in situ* hybridization, *in situ* PCR for detection of integrin RNA and immunofluorescence detection of antibody-tagged integrin protein. A decrease of about 25 to 100% in α1 integrin expression can indicate that early changes of diabetic nephropathy are occurring and can be used to identify patients that have an increased risk of developing diabetic nephropathy. A decrease in α1 integrin expression is compared to the level of α1 integrin expression in cells from age matched non-diabetic controls.

For detection and quantitation using *in situ* hybridization, the following method is preferred: a detectably labeled probe that is complementary to and/or hybridizes to all or a portion of nucleic acid sequences encoding all or a portion of α1 integrin subunit is utilized. A radioactively labeled probe preferably has a specific activity of about $2x10^8$ to $1x10^9$ dpm/μg. *In situ* hybridization on cells such as kidney tissue can be conducted as follows. 5μm tissue sections, fibroblasts and/or blood cells on silane-coated slides are further fixed in fresh 4% paraformaldehyde for 10 min. The slides are then pretreated with 0.2N HCl for 20 min., 0.05 M Triethanolamine (TEA, Sigma) for 15 min, 0.005% digitonin for 5 min., 3 μg/ml proteinase K (Sigma) for 15 min. at 37°C,

and 0.3% acetic anhydride - 0.1M TEA for 10 min. Hybridization is performed at 50°C overnight in 50% formamide, 0.6 M NaCl, 1xDenhardt's, 0.17 μg/ml human COT^{RT} DNA (GIBCO/BRL), 1 mg/ml poly A (Boehringer Mannheim), 10% (W/V) Dextran sulfate (Sigma), 0.1 M dithiothreitol (DTT, Boehringer Mannheim), 1 mM EDTA, 0.1 mM aurinitricarboxylic acid (ATA, Sigma) and S^{35}-dCTP labeled cDNA probe. The following day, the slides are washed in 2x SCC-0.05% SDS for 60 min. at 55°C; further washed in the high stringency washing buffer containing 50% formamide, 0.6 M NaCl, 1 mM EDTA, 5 mM DTT and 10 mM Hepes for 4 days at room temperature. After 4 days, the slides are rinsed in 2x SCC and the slides are dehydrated in graded ethanol with 0.3 M ammonium acetate, then dipped in Kodak NTB-2 emulsion and exposed for 5 days at 4°C. After development, the slides are stained with hematoxylin-eosin (Surgipath Canada, Inc., Winnipeg, Canada) and mounted. The silver grain number per cell are used to quantitate the result of *in situ* hybridization. About 10-20 glomeruli and a similar number of tubules are examined per patient.

A probe of the invention hybridizes to and is complementary to and/or all or a portion of a nucleic acid sequence encoding α1 integrin as long as the probe specifically detects α1 integrin expression. Probes can be designed using a known sequence such as the rat α1 integrin sequence as shown as Figure 2 in Takada and Hemnlev, *J. Cell Biol.* 109:397-407 (1983) or by the use of commercially available programs and are capable of binding to rodent or human α1 integrin but are not capable of binding to other proteins including other proteins having regions homologous to α integrins when tested under identical hybridization conditions. Examples of other proteins that have homologous regions to α integrins include those proteins identified using a gene bank search, such as GenBank, or the like, or in publications related to α1 and α2 subunits (for example, see Ignatius, et al. *J. Cell Biol.* 111:709-720, 1990 listing proteins with homologies to the α1-subunit).

The probe can be about 15 nucleotides long up to a full length probe of about 4kb. The probes are preferably 100% complementary to the nucleic acid encoding α1 integrin however some mismatches can be present depending on the length of the probe. About 1 to 3 mismatches in a probe of about 20 to 30 nucleotides long can be present as long as hybridization conditions are adjusted to account for mismatches. Hybridization conditions can be adjusted to take into account mismatches in accord with known

principles as described in Sambrook et al., A Guide to Molecular Cloning, Cold Spring Harbor NY (1989).

A specific example of a nucleic acid sequence encoding α1 integrin is the rat α1 integrin sequence shown as Figure 2 in Ignatius et al., *J. Cell. Biol.* 111:709-720, 1990, (SEQ ID NO:1) and the protein sequence encoded by α1 integrin is provided as SEQ ID NO:2. A DNA sequence encoding α1 integrin can be obtained from a rat pheochromocytoma cell line PC12 as described by Ignatius et al., *J. Cell. Biol.* 111:709 (1990). Briefly, a cDNA library can be prepared from rat pheochromocytoma PC12 in a lambda vector. The sequence can be identified and/or amplified using probes or primers designed from the known sequences using standard methods as described in Sambrook et al., (*supra*). Once the sequence is subcloned it can be confirmed by sequence analysis and/or by screening with antibodies specific for α1 integrin. Other DNA sequences encoding α1 integrins can be identified and isolated using probes and primers derived from the known sequences.

A preferred probe is a 3.9 kb fragment from the 5' end through the EcoR1 site near base 3900 including the sequence as shown in Figure 2 of Ignatius et al. *(supra)*. Smaller fragments that can form probes can readily be prepared with restriction enzymes or derived by automated or manual oligonucleotide synthesis techniques, by PCR, or by other methods also known in the art. The probes are preferably detectably labeled with a radioactive nucleotide using standard methods.

Other methods utilizing probes for detection of α1 integrin expression can also be utilized using standard methods such as Northern Blot Analysis and the like as described in Sambrook et al., cited *supra*.

Primers can also be designed based upon the sequence of rat α1 integrin sequence. This invention also contemplates using primers and nucleic acid sequences from the human α1 integrin sequence provided by Briesewitz, et al. (*J. Biol. Chem.* 268(4):2989-96, 1993). Primers can be designed using a known sequence using commercially available computer programs. Primers typically are complementary to and/or hybridize to a 5' region and/or a 3' region of the nucleic acid sequence encoding the protein of interest. The primers can be used to amplify all or a portion of DNA or cDNA encoding α1 integrin. Primers can be used to make probes and to detect expression levels of α1 integrin. Primers preferably have at least 15 nucleotides that are

100% complementary to the nucleotide sequence selected. The primers can also have additional sequences preferably at the ends of the primer that include restriction enzyme sites and the like that are not complementary to the nucleic acid sequence to be amplified. Primers are preferably about 15 to 50 nucleotides long and can be prepared by automated synthesis.

The primers can be used to detect the level of α1 expression in cells. RNA from cells is extracted and reverse transcribed using standard methods. Primers that are complementary to and can hybridize to a DNA sequence encoding α1 integrins are utilized to amplify the cDNA. A decrease in the level of PCR product can be determined in comparison to the amount of PCR product obtained from control cells.

One method of utilizing PCR to detect α1 integrin expression is *in situ* PCR. A method for PCR *in situ* hybridization is described in PCR *In Situ* Hybridization Protocols and Applications, J. Novo ed., "PCR *In Situ* Hybridization", pp. 157-183. Briefly, tissue sections, fibroblasts and/or blood cells (about 5 μm) are placed on silane-coated glass slides. After removing paraffin, the slides are treated with trypsinogen (2mg/ml) in 0.01N HCl for 10 minutes and then trypsinogen inactivated in 0.1M Tris HCl (pH 7.0) solution. The slides are washed sequentially in 90% and 100% ethanol, two times for 1 minute each and air dried. Aliquots of reaction mixture containing 0.15 units/ml *Taq* DNA polymerase and specific primer pairs for α1 integrin are added to the tissue section and then overlaid with siliconized glass coverslips. The slides are placed in the heat-sealable plastic bags and 4-5ml mineral oil is added. After removing air, the bag is heat-sealed and placed in the thermal-cycling oven for 40 cycles. After thermal-cycling, the slides are washed twice in chloroform for 2 minutes. The coverslips are removed and the slides are dipped briefly in fresh chloroform. After washing in PBS for 5 minutes, the slides are dehydrated and air-dried. The slides are dipped in NTB2 nuclear emulsion (Kodak) and exposed in the dark for 7 days. After development, the slides are *counterstained* with hematoxylin-eosin.

A change in the level of α1 integrin protein expression can also be detected by using immunofluorescence. (Unless otherwise specified as "protein expression", the term "expression" used herein generally refers to RNA expression.) Sections of tissue samples, fibroblasts and/or blood cells can be stained with antibodies specific for α1 integrin. It is preferable that antibodies are monoclonal antibodies and are antibodies

that do not substantially cross-react with other α integrin subunits. Antibodies to α1 integrin can be made by standard methods such as described in Wayner EA and WG Carter, 1987, *J. Cell Biol.* 121(5):1141-1152. Antibodies specific to α1 integrin include the SR84 and TS2/7 antibodies. Information related to these antibodies is provided in Examples 1 and 3. A decrease in the level of immunofluorescence can be observed and quantitated using standard methods. A decrease of about 25 to 100% of α1 integrin expression may be used to identify patients that have a greater risk of developing diabetic nephropathy. A decrease in α1 integrin expression is compared to the level of α1 integrin expression in age-matched nondiabetic controls.

The preferred method of the invention involves comparing the level of expression of α2 integrin to the level of expression of α1 integrin. Under high glucose conditions, a decrease in the level of α1 expression is seen as well as an increase in the level of α2 expression in mesangial cells. It is believed that patients at greater risk for nephropathy or other complications associated with diabetes will exhibit an increase in α2 expression and a decrease in α1 expression. A change of about 15 to 100%, and preferably of about 25 to 100%, of α2 integrin expression as well as a change of about 15 to 100%, and preferably of about 25 to 100%, of α1 integrin expression is believed to be indicative of patients with a greater risk of developing diabetic nephropathy.

Integrin expression is associated with a variety of cell types in a variety of locations throughout the body, therefore it is possible that altered levels of integrin expression will also be identified in diabetic associated retinopathy, atherosclerosis and select diabetic neuropathies.

The expression of integrin subunits, preferably of α1 and α2 integrin subunits, is detected and/or quantitated in tissue samples, fibroblasts and/or blood cells from diabetic patients. The preferred methods are those that allow detection of gene expression in a small amount of cells or tissue.

The expression of α2 integrin can be detected using *in situ* hybridization. The conditions for *in situ* hybridization are the same as those described previously. A probe specific for nucleic acid sequences encoding α2 integrin can be prepared using standard methods as described in Sambrook et al., cited *supra*. The probes are complementary to and/or hybridize to all or a portion of a nucleic acid sequence encoding α2 integrin. As described for α1 integrin, the probe to detect α2 integrin can hybridize to a portion of a

nucleic acid sequence as long as the probe specifically detects a sequence encoding α2 integrin. Nucleic acid sequences can be DNA, cDNA, or RNA. It is preferred that the probe hybridize to RNA or cDNA.

A specific example of nucleic acid sequence encoding α2 integrin is shown in Figure 2 of Takada and Hemler, *J. Cell Biol.* 109:397 (1989). (SEQ ID NO:3). DNA sequence encoding human α2 integrin can be isolated as described in this reference. The protein encoded by SEQ ID NO:3 is provided in this disclosure as SEQ ID NO:4. Nucleic acid sequences encoding α2 integrin can be obtained from human lung fibroblasts and/or human endothelial cells. Preferably DNA libraries from endothelial cells can be prepared and nucleic acids encoding α2 integrin identified and/or amplified using probes and primers derived from the sequence of α2 integrin, e.g., as shown in Figure 2 of Takada et al. (*supra*). If primers are selected, DNA sequences can be amplified using the polymerase chain reaction and then subcloned. Clones that are positive by hybridization to a probe specific for DNA sequences encoding α2 integrin (see Examples 1 and 3) or that express proteins that are positive by reacting with an antibody specific to α2 integrin such as P1H5 are selected. A DNA sequence encoding α2 integrin can be confirmed by DNA sequencing in comparison to the known α2 sequence, as shown in Figure 2 of Takada et al. (*supra*).

A probe of the invention hybridizes to and is complementary to and/or hybridizes to all or a portion of a nucleic acid sequence encoding α2 integrin as long as the probe specifically detects α2 integrin expression. Probes can be designed using a known sequence such as shown in Figure 2 of Takada et al. (*supra*) by the use of commercially available programs.

The probe can be about 15 nucleotides long up to a full length probe of about 5Kb. The probes are preferably 100% complementary to the nucleic acid encoding α2 integrin however some mismatches can be present depending on the length of the probe. About 1 to 3 mismatches in a probe of about 20 to 30 nucleotides long can be present as long as hybridization conditions are adjusted to account for mismatches. Hybridization conditions can be adjusted to take into account mismatches in accord with known principles are described in Sambrook et al., A Guide to Molecular Cloning, Cold Spring Harbor NY (1989).

A preferred probe is a 1.8 fragment kb from the 5' end through the EcoR1 site near base 1800 of the sequence shown in Figure 2 of Takada et al. (*supra*). Other probes can be derived from this fragment or from the full length sequence by use of restriction enzyme digestion. Probes can also be prepared by automated synthesis or by PCR. Probes are preferably detectably labeled with a radioactive nucleotide using standard methods.

Probes specific for α2 integrin expression can then be utilized in methods of detecting α2 integrin expression in various cell types. The preferred method is by use of *in situ* hybridization or PCR-*in situ* hybridization on kidney as well as other tissues. The method utilized for *in situ* hybridization has been described previously (Takada and Hemler, *supra*). The method for PCR *in situ* hybridization has been described for α1 integrin. Other methods utilizing probes for detection of α2 integrin expression can also be utilized using standard methods such as Northern Blot Analysis, and the like, as described in Sambrook et al. cited *supra*.

Primers can also be designed based upon the known DNA sequence encoding human α2 integrin. Primers can be designed from a known sequence such as shown in Figure 2 of Takada et al. (*supra*), using commercially available software. Primers typically are complementary to and/or hybridize to a 5' region and/or a 3' region. The primers can be used to amplify all or a portion of DNA or cDNA encoding α2 integrin. Primers can be used to make probes and to detect expression levels of α2 integrin. Primers preferably have at least 15 nucleotides that are 100% complementary to the nucleotide sequence selected. The primers can also have additional sequence preferably at the ends of the primer that include restriction enzyme recognition sites and the like. Primers are preferably about 15 to 50 nucleotides long and can be prepared by automated synthesis.

Primers can be used to detect the level of α2 integrin expression in cells. Nucleic acids, preferably RNA, from cells from diabetic patients are extracted and reverse transcribed using a standard method. Primers that are complementary to and can hybridize to a cDNA sequence encoding α2 integrin are utilized to amplify the cDNA. An increase in the level of PCR product can be determined in comparison to the amount of PCR product obtained from control cells.

A change in the level of α2 integrin protein expression can also be detected by using immunofluorescence. Sections from kidneys and/or other tissues, skin fibroblasts and/or blood cells can be incubated with antibodies specific to α2 integrin. It is preferable that the antibodies are monoclonal antibodies and are antibodies that do not crossreact with other α integrin subunits. Antibodies to α2 integrin can be made by standard methods such as described in Wayner EA and WG Carter, 1987, *J. Cell Biol.* 121(5):1141-1152. Antibodies specific for α2 integrin include P1H5. An increase in the level of immunofluorescence can be observed and quantitated using standard methods such as flow cytometry. An increase of about 25 to 100% of α2 integrin expression can be used to identify patients that have a greater risk of developing diabetic nephropathy. An increase in α2 integrin expression is compared to α2 integrin expression in nondiabetic control cells.

An increase in α2 integrin expression alone can also be used to identify a patient that may have a greater risk of developing diabetic nephropathy. An increase in α2 expression can be determined as described using the methods described above. An increase of about 25 to 100% in α2 integrin expression may indicate a patient who has an increased risk of developing diabetic nephropathy.

Although an increase of α2 integrin expression or a decrease of α1 integrin expression alone can be utilized to identify patients at greater risk for developing diabetic nephropathy, a preferred method is to detect changes in both α1 and α2 integrin expression. It is believed that an increase in α2 integrin expression and a decrease in α1 integrin expression identifies patients that are at greater risk of or are showing early symptoms of diabetic nephropathy.

In one step of the method, the level of α2 to α1 integrin is compared. The level of α1 integrin expression can be detected and/or quantitated using the methods described previously. The level of α1 and α2 integrin expression can be quantitated on two different cell samples such as two sections of the same tissue sample. About 10-20 glomeruli and tubules are examined. On one cell sample containing the same type of cells, α2 integrin expression can be quantitated and on a second cell sample with the same type of cells, α1 integrin expression can be quantitated. Alternatively, the level of α1 and/or α2 integrin expression can be determined using the same cell sample if the agent used to detect α1 expression is detectably labeled with a first detectable label and

the agent used to detect α2 expression is detectably labeled with a second detectable label. The first detectably labeled agent and the second detectably labeled agent are agents selected that can be detected and/or quantitated in the presence of one another.

In a preferred version, kidney tissue sections taken from diabetic patients are fixed in formalin and then treated with HCl and proteinase K. A first probe specific for α1 integrin is a 3.9 kb fragment from 5' end through EcoR1 site near base 3900 probe including a sequence as shown in Figure 2 of Ignatius et al. (*supra*). This probe is labeled with ^{32}P or 35S or other suitable labels known in the art including, but not limited to, fluorescent labels, biotinylated labels, or other radio labels and the like. The probe is incubated with the section as described previously. A second section taken from the same tissue sample is treated in the same manner but incubated with a probe specific for α2 integrin expression. In a preferred embodiment, a probe specific for α2 integrin expression is a 1.8 kb fragment from 5' end through EcoR1 site near base 1800 that includes a sequence as shown in Figure 2 of Takada et al. (*supra*). Both probes are labeled with ^{32}P or ^{35}S. The probe is incubated with the section overnight at 50°C and then for 4 days at room temperature. The sections are then developed for autoradiography. The number of grains per cell are counted for about 10-20 glomeruli and tubules. The total counts for α2 integrin expression vs. α1 integrin expression are compared. An increase of about 40% in α2 integrin and a 30-40% decrease of α1 integrin may indicate a patient is at greater risk for developing diabetic nephropathy.

In an alternative version, the level of expression of α2 integrin is compared with the α1 expression which can be determined using *in situ* PCR or competitive reverse transcriptase PCR. Primers specific for α1 and α2 integrin expression can be prepared as described previously. For competitive reverse transcriptase PCR, RNA extracted from different cell types obtained from diabetic patients will be reverse transcribed to generate cDNA. The cDNA will be mixed with the various concentrations of competitive template amplified by the PCR method. After degradation of competitive cDNA with restriction enzyme, amplified cDNA will be subjected to electrophoresis in 2% agarose gel, electrotransferred to a nylon membrane, UV cross-linked to the membrane and hybridized with a ^{32}P-labeled probe. Autoradiographs will be used to quantify the label bound to the cDNA using amount of label bound to samples containing target cDNA alone as compared to samples also containing competitor

cDNA to arrive at the target cDNA concentration. For *in situ* PCR, a method has been described previously. The change in α1 and α2 integrin expression can be quantitated by counting the number of grains per cell in control vs. diabetic cells.

Optionally, for each of the detection methods for α integrin subunits, the level of integrin subunit expression can be compared to expression of a control. The control is selected to be a protein expressed at the same levels in both normal and diabetic cells. The control protein is also selected to be one that is expressed at sufficient levels for easy detection and quantitation. The level of expression of α1 and α2 integrin expression can each be compared to that of the level of the control RNA expression in the cells. The level of RNA expression of α1 integrin or α2 integrin can be divided by the level of expression of the control RNA to normalize the values to the level of control expression in a particular cell sample. The level of expression of the control protein is detected and quantitated using the same method as α1 or α2 integrin expression. The preferred control protein is a cell surface HLA determinant.

Optionally, the levels of α3, α5, or beta-1 integrin subunit expression can be analyzed as described above. The level of α3, α5, or beta-1 integrin expression in cells such as kidney tissue can be detected and quantitated as described for α1 and α2 integrin expression including *in situ* hybridization, *in situ* PCR, immunofluorescence and the like. Other cell types can be analyzed as described above, including fibroblasts and blood cells. Antibodies specific for α3, α5, and beta-1 can be prepared as described by Wayner et al. cited *supra*.

A DNA sequence encoding α3 integrin has been described in Takada et al., *J. Cell Biol.* 115:257 (1991). A probe specific for cDNA sequence encoding α3 integrin subunit is a 1.4Kb Sal I fragment containing 5' untranslated and amino terminal coding sequences for α3 subunit of integrin. DNA sequences encoding α3, α5, and beta-1 integrin can be utilized to form primers and probes as described previously.

The level of α3, α5, or beta-1 integrin expression is increased about 15 to 100% compared with cells from age matched nondiabetic controls. It is believed that an increase in α3, α5, or beta-1 integrin subunit expression may also identify patients that have an increased risk of developing diabetic nephropathy or that have early signs of diabetic nephropathy.

This invention also relates to methods for detecting alterations in integrin subunit expression, particularly α1 and/or α2 integrin subunit expression by obtaining a cell sample from a patient, processing the sample to detect alterations in integrin subunit expression as compared to integrin expression in samples from age matched normal controls, detecting levels of integrin expression and determining if these levels are altered relative to controls.

This method is useful for predicting individuals at risk for developing pathologies associated with altered cell matrix deposition, including but not limited to renal nephropathy. In preferred embodiments of this invention, the tissues used to detect altered α1 and/or α2 integrin expression include kidney biopsies, skin biopsies and blood cells including polymorphonuclear cells, monocytes, and other cells expression integrin subunits. Biopsied tissue can be further separated into its cellular components or processed as tissue sections for *in situ* hybridization techniques, and/or for immunodiagnostic techniques including immunofluorescence and immunoperoxidase staining.

The cellular components of the biopsied tissue can be cultured for *in vitro* studies including Northern procedures, PCR techniques, immunofluorescent techniques and/or in situ hybridization techniques. Alternatively, cells can be separated and analyzed by flow cytometry, immunofluorescence, processed for PCR or for any of a variety of techniques discussed throughout this disclosure.

While blood cell components are preferably separated from the whole blood sample using methods well known in the art. Individual cells are separated, where necessary, using techniques such as those of Ng, et al. (*supra*), and Baron, et al. *Clin. Sci.* 37:205-219, 1990. Preferably the samples are tested using *in situ* hybridization methods. Where the amount of tissue available is fairly small, PCR-enhanced *in situ* hybridization can be used.

The present invention is also directed to a kit to detect alterations in integrin subunit expression, particularly α1 integrin and/or α2 integrin subunit expression in a patient sample. A variety of kits are contemplated to encompass a variety of methods. These kits optionally include reagents to process a tissue or cell sample for the technique employed by that particular kit. By example, a kit for PCR or PCR enhanced *in situ* hybridization can include reagents to process the cell sample or section and

isolate the RNA (for PCR). It will also contain suitable primers to amplify the target sequence and additional probes, if necessary, to detect the desired nucleic acid fragments as well as buffers and reagents for the polymerase chain reaction and the buffers and emulsions required to develop the silver granules, and the like, for *in situ* hybridization methods. Other kits can alternatively include reagents for immunofluorescence using antibodies to the integrin subunits and/or probes, primers and reagents for modifications of *in situ* or PCR *in situ* hybridization methods.

All publications and patent applications in this specification are indicative of the level of ordinary skill in the art to which this invention pertains. All publications and patent applications are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated by reference.

It will be apparent to one of ordinary skill in the art that many changes and modifications can be made in the invention without departing from the spirit or scope of the appended claims.

Example 1
Effect of High Glucose on the Synthesis and Cell Surface Expression of Integrin Receptors by Cultured Mesangial Cells

Cell lines and culture conditions

Human mesangial cells (HMC) were isolated from 19-22 week old fetal kidney tissue or adult tissue as previously described (Striker and Striker, *J. Lab. Invest.* 53(2):122-131, 1985). Cells were cultured at 37°C in an environment of 95% air and 5% $C0_2$ and in media composed of MEM (Sigma, St. Louis, MO) containing 5 or 25 mM glucose, 20% FBS, 15mM Hepes, penicillin (100 U/ml), streptomycin (100mg/ml), and amphotericin (25mg/ml). Cells were cultured in the two different conditions for at least two passages before they were used for experiments. Cells were released from their tissue culture flasks for passaging or for use in experiments, by washing twice with 1 mM EDTA in HBSS and then treating with 0.05% trypsin and 1 mM EDTA in HBSS for 1 min. Cells between passage 4 and 9 were used in experiments.

The cells were grown in T-75 flasks until 75-80% confluent. For the adhesion and immunoprecipitation analyses, cells were metabolically labeled for 18 hours with 0.5 mCi of [^{35}S]-methionine per T-75 flask. [^{35}S]-methionine was obtained from Du Pont/NEN, Boston, MA.

Monoclonal antibodies (Mabs) to integrin receptors

Mabs to the integrin receptors α3 (P3D11), α5 (P3D10) and β1 (P5D2) can be produced as previously described (Wayner et al., *J. Cell. Biol* 121(5):1141 (1993)) and are available from EA Wayner, Fred Hutchinson Cancer Center, Seattle, WA. The antibodies were characterized by sequential immunoprecipitation with known Mabs directed against these integrin receptors (P1B5, P1D6, P4C10) available from EA Wayner. Other Mabs α2 (P1H5), α4 (P4G9) and β2 (P4H9) were previously described (Wayner et al., cited *supra* 1993) and are available from EA Wayner, Fred Hutchinson Cancer Center, Seattle, WA. TS2/7 was provided by Dr. Martin Hemler (Dana Farber Cancer Institute, Boston, MA).

SR84 supernatant was used as a function-blocking anti-α1 Mab in inhibition experiments. SR84 is available from Dr. D.O. Clegg (Univ. of California, Santa Barbara, CA). (α6) G0H3 was purchased from AMAC Inc., Westbrook, ME. In addition monoclonal antibodies to α1 and α2 integrin were obtained from Telios Pharmaceuticals (San Diego, CA). Hybridoma culture supernatant or ascites fluid were used for immunoprecipitation, flow cytometry and inhibition experiments. A Mab directed to a cell surface HLA determinant was used as a negative control (W6/32, HB95: American Type Culture Collection, Rockville, Maryland, USA). W6/32 bound to the surface of cultured mesangial cells but did not influence adhesion of cIV. SP2 myeloma culture supernatant was also used as a control.

Immunoprecipitation analysis of integrins from mesangial cell membranes

Mesangial cells metabolically labeled with [^{35}S]-methionine were detached from flasks by treatment with trypsin (Sigma) for 2 minutes, washed three times with phosphate-buffered saline (pH 7.4) and resuspended in PBS containing protease inhibitors (1 mM PMSF and 1 mM NEM). The radiolabeled cell membrane proteins were solubilized by adding lysis buffer (1% Triton X-100, 1 mM Calcium, 1 mM

PMSF, 1 mM NEM and PBS at pH 7.4) and incubating for 60 minutes at 4°C. Insoluble material was separated by centrifugation at 10,000 rpm for 30 minutes.

The supernatant was transferred and 10 µl was tested for radioactivity ($\geq 10^7$ cpm/per antibody being assayed was considered to be adequate for immunoprecipitation). The lysate was precleared once with fetuin-agarose which was removed by centrifugation at 10,000 rpm for 15 minutes. This was followed by three preclears with protein A agarose bound to rabbit anti-mouse IgG, the last preclear was done overnight.

For immunoprecipitation, the cell lysate (equal counts of lysate for cells in 5 and 25 mM glucose were used) was incubated with the monoclonal antibodies to be tested, pre-bound to rabbit anti-mouse protein A-agarose. Myeloma culture supernatant was used as a negative control. Anti-HLA antibody (W6/32) was used as a control for loading. After an overnight incubation at 4°C, the agarose beads were washed five times and bound material was eluted by boiling for 5 minutes in SDS.

The eluted material was analyzed by loading lysate from each permutation on a 7.5% non-reducing SDS-PAGE gel and labeled proteins were visualized by autoradiography. The fluorograms were scanned with a Macintosh Quadra 840 computer using the NIH Image 5.1 Program, and the optical density of the bands was red after subtracting the background. The O.D. was corrected using the lanes immunoprecipitated with W6/32. Immunoprecipitation assays were performed three times for each growth condition of mesangial cells.

Immunoprecipitates were obtained with anti-integrin monoclonal antibodies from detergent extracts of metabolically labeled human kidney mesangial cells grown in 5 (low) or 25 mM (high) glucose. Equal counts of membrane proteins were immunoprecipitated to compare the level of integrin receptors of mesangial cells under the two growth conditions of low or high glucose levels.

Cells grown in 25 mM glucose have a higher specific activity of labeling than cells in 5 mM glucose. To overcome this difference and permit a comparison of the band intensity on immunoprecipitation equal counts of cell lysate from the two populations were immunoprecipitated with the antibody. Densitometry and statistical analysis of three experiments was performed, the data normalized to the HLA control and expressed as an O.D. ratio of cells grown in high glucose (HG) to cells grown in

low glucose (LG), for three experiments, with (LG = 1). Cells were labeled with [^{35}S]-methionine, the cells were harvested, and solubilized. Samples were incubated with antibody and equal counts of cell lysate from the two cell populations were immunoprecipitated with equal amounts of antibody.

The control indicated that there were comparable amounts of cell surface HLA determinant precipitated from each sample. W6/32, a Mab to cell surface HLA determinant was used as a negative control. Other antibodies used included an anti-α1 antibody (TS2/7) and an anti-α2 antibody (P1H5). In total 5 mM and 25 mM glucose exposed cell extracts were immunoprecipitated side by side 3 times.

The α1 subunit band was clearly discernible at 180 kD in cell samples exposed to 5 mM of glucose and was associated with a β1 band (116 kD). No α1 band could be seen in the 25 mM treated cell sample. In contrast, the α2 subunit band was more prominent in cell samples exposed to 25 mM glucose and appeared as a band at 130 kD. The 130 kD α2 band was present in 5 mM glucose but was significantly less intense than the 25 mM glucose treated samples.

The cell lysates were also incubated with the following antibodies including: SP2 myeloma culture supernatant; anti-β1 (P5D2), anti-β2 (P4H9), anti-α2 (P1H5), anti-α3 (P3D11), anti-α4 (P4G9), anti-α5 (P3D10) and anti-α6 (G0H3). Results were interpreted from three independent experiments. Immunoprecipitation of α3-α6 and β1 integrin subunits was performed on cells from the two growth conditions. Subunits α4 and α6 were not detected in either cell population. The antibody to the β1 subunit precipitated a 116 kD protein, the β1 subunit, and also a precursor β1 band at 105 kD. The α3 and α5 subunits were seen at ≈ 130 kD with the associated β subunit at 116 kD, in both cell populations.

Flow cytometry

Cell surface expression of integrin subunits by cultured human mesangial cells was evaluated by indirect immunofluorescence staining and flow cytometry. Mesangial cells were released with trypsin, washed and resuspended in FACS buffer (HBSS, 2% goat serum, 0.02% sodium azide). An equal number of cells, 2 x 10^5 were added to each vial.

The cells were incubated with primary antibody for one hour at 4°C and washed once with 1 ml FACS buffer. The secondary antibody was then added in a total volume of 0.5 ml FACS buffer and incubated for 30 minutes at 4°C. The cells were again washed in 1 ml of FACS buffer and resuspended in 0.5 ml of 2% formaldehyde.

The data was analyzed using CONSORT 30 software on a FACScan (Becton Dickinson, Mountain View, CA). Positive fluorescence was determined on a four decade log scale and fluorescence (log F1) was expressed as the mean channel number of 5,000 cells. Cell surface expression experiments were performed in duplicate with each antibody, at least three times with each growth condition of mesangial cells.

Densitometric scanning of the fluorograms generated from metabolically labeled cells indicated that the synthesis of the β1 (12%), α3 (14%) and α5 (19%) were moderately increased upon growth in 25 mM glucose. Growth in 25 mM glucose dramatically decreased synthesis of the α1 subunit (39% reduction in intensity) while synthesis of α2 was considerably increased (42%).

These changes in metabolic activity were paralleled by a similar change in the cell surface integrin phenotype of mesangial cells grown in high glucose. To assess the effect of different glucose concentrations in the medium on the levels of mesangial cell surface integrin receptor expression cells in each glucose treatment population were stained for immunofluorescence and processed for flow cytommetry. Mean channel fluorescence (MCN) values of integrin subunit expression were obtained from 3 experiments. Within each experiment the ratio of MCN for cells grown in high glucose (HG) to cells grown in low glucose (LG), denominator = 1 was calculated.

Cell surface expression of the following integrin subunits was increased by growth in high glucose: β1 (24%), α2 (26%), α3 (18%), and α5 (19%). The decrease in the synthesis of α1 was reflected in a concomitant decrease in cell surface expression (33% reduction in specific staining). The α4 and α6 subunits were not detectable in cultured mesangial cells either by immunoprecipitation or flow cytometric analyses.

Mesangial cells grown in high glucose (for at least 2 passages) were returned to control media (5 mM glucose), again for at least 2 passages. A flow cytometric analysis of these cells revealed a reversion to "low glucose" type. The expression of α2, α3, α5 and β1 were decreased while the expression of α1 increased (data not shown).

Example 2
Adhesion of Cultured Mesangial Cells to
Type IV Collagen (cIV): Effect of High Glucose

Cell adhesion to collagen IV (cIV)

The cells were detached from culture flasks by incubation with trypsin 0.05% and EDTA 0.02% for two minutes at 37°C, then washed twice with DMEM and resuspended to the appropriate concentration in binding buffer (DMEM, 25 mM HEPES, 2 mg/ml BSA at pH 7.4). 48 or 96 well plates were coated overnight at 29°C with cIV in serial dilutions starting from 100 μg/ml (5 μg/96 well or 20 μg/48 well). Under these conditions approximately 50% of the cIV adhered. To block the remaining reactive sites the plates were treated with 200 μl of BSA at 2 mg/ml for 2 hours at 37°C. 50 μl of suspension containing 5000 cells (96 well plates) or 100,000 cells (48 well plates) was added per well. The plates were incubated at 37°C in a humidified incubator for approximately 30 minutes. The non-adherent cells were removed by washing three times with binding buffer and then 100 μl of "lysis" buffer (0.5 NaOH, 1% SDS in distilled water) was added to each well for 30 minutes at 60°C. The lysate was transferred to scintillation vials and counted. The data was expressed as a percentage of the total input cpm. Cell adhesion assays were performed in triplicate, at least three times for each growth condition.

Cells grown in medium containing 25 mM glucose adhered significantly better than cells in 5 mM glucose. Adhesion increased with coating concentration of cIV and was saturated at 25 μg/ml for both cell populations.

Inhibition of cell adhesion with monoclonal antibodies

Since growth in high glucose appeared to alter the synthesis and expression of the integrin receptors α1β1 and α2β1 which have been reported to be involved in cell adhesion to collagen, (Wayner and Carter, *J. Cell. Biol.* 105:1873 (1987)), we examined the effects of glucose on the ability of mesangial cells to adhere to cIV.

Monoclonal antibody inhibition of ^{35}S-methionine labeled human mesangial cells grown in 5 mM glucose to cIV was assessed. Briefly, 96 or 48 well plates were coated with 50 or 200 μl of cIV at 2.5 μg/ml, overnight at 29°C. The plates were incubated with 2% BSA in PBS to coat remaining reactive sites on plastic for 2 hours,

and then hybridoma culture supernatant or ascites containing 10 μg/ml of antibody were added to each well, followed immediately by the cells. After 30 minutes non-adherent cells were washed off and adherent cells were quantitated. Results were obtained from 3 experiments. SP2 myeloma culture supernatant of W6/32 were used as negative controls. A quantitative ELISA was used to determine the concentration of antibody in the hybridoma culture supernatant or ascites.

In each case, the concentration of monoclonal antibody (Mab) was determined relative to a standard curve generated with an isotype-matched control mouse IgG. The concentration of antibody required to saturate the binding sites on human mesangial cells was determined by flow cytometry. The concentration of the antibodies used in the inhibition assays were well above the saturating concentration as determined by flow cytometry. Data were expressed as the percent of maximal binding observed in the presence of W6/32 antibody. Inhibition experiments were performed at least three times, in triplicate, for each growth condition with the various antibodies.

Mesangial cells grown in high glucose (25 mM) adhered better to cIV than cells grown in low glucose (5 mM). Results indicated that adhesion increased with coating concentration of collagen IV and saturated at about 25 μg/ml for both cell populations.

In order to examine the activity of collagen receptors expressed by mesangial cells grown in high glucose, we performed adhesion experiments in the presence of well characterized neutralizing antibodies directed to various β1 integrin subunits. A panel of antibodies was used all of which have been reported to inhibit the adhesion of cells to various substrates (Wayner and Carter, cited *supra*, 1987; Wayner et al., cited *supra*, 1993). Antibodies were used at saturating concentrations as determined by immunofluorescence staining and flow cytometry. In the competition experiments, the following criteria were selected to promote half-maximal binding of mesangial cells: 2.5 μg/ml cIV and a short term assay (less than 30 min). The ability of neutralizing Mabs to inhibit mesangial cell adhesion to cIV was examined in low (5 mM) or high glucose (25 mM) containing media.

To test Mab-mediated adhesion inhibition of mesangial cells grown in 5 mM glucose or 25 mM glucose to collagen IV, ^{35}S-methionine labeled human mesangial cells were seeded in 48 well plates (100,000 cells/well) coated with 200 μl cIV (2.5 μg/ml, overnight at 29°C). Mab's anti-α1, SR84, anti-α2, P1H5, anti-β1, P5D2 and

SR84 and P1H5 together, were added to the wells before seeding with cells. Adhesion in the presence of W6/32 was used as a control. After 20 minutes non-adherent cells were washed out and adherent cells quantitated. The data was expressed as a percentage of the binding in the presence of W6/32. and the two cell populations were normalized by using the binding in the presence of HLA antibody to represent 100% and the inhibition by other antibodies was calculated as a percentage of binding in the presence of HLA.

The results indicated that the $\alpha 1\beta 1$ integrin receptor had a reduced role ($^*p < 0.001$) for cells grown in 5 mM glucose as compared with 25 mM glucose. Of the antibodies examined, only Mabs directed to the $\alpha 1$ (SR84), $\alpha 2$ (P1H5) or $\beta 1$ (P5D2) integrin subunits inhibited the binding of mesangial cells to cIV. When mesangial cells were grown in either low or high glucose, adhesion to cIV could be almost completely inhibited with Mabs to $\beta 1$ (P5D2) or a combination of $\alpha 1$ (SR84) and $\alpha 2$ (P1H5).

The relative effects of the neutralizing Mabs directed against the $\alpha 1$ and $\alpha 2$ subunits varied depending on whether mesangial cells were grown in low or high glucose. In 5 mM glucose the Mab to the $\alpha 1$ subunit of integrins resulted in more inhibition ($\approx$ 50%) than in 25 mM glucose ($\approx$ 20%) ($p < 0.001$). This is consistent with the presence of significantly more $\alpha 1$ integrin on the surface of cells grown in 5 mM glucose. Alternatively, in 5 mM glucose the Mab to the $\alpha 2$ subunit resulted in less inhibition ($\approx$ 60%) than in 25 mM glucose ($\approx$ 75%) ($p < 0.001$). Mab's against the $\alpha 3$, $\alpha 4$, $\alpha 5$ and $\alpha 6$ subunits did not inhibit adhesion (data not shown).

These data demonstrate that under low glucose growth conditions, mesangial cells use $\alpha 1\beta 1$ and $\alpha 2\beta 1$ integrins to bind cIV coated surfaces. However, cells grown in high glucose, appear to rely more on the $\alpha 2$ subunit complexed with $\beta 1$. The results of these functional studies are consistent with the observed alterations in the integrin cell surface phenotype discussed in Example 1.

Example 3
Localization of $\alpha 1\beta 1$ and $\alpha 2\beta 1$ Integrin Receptors

Localization of $\alpha 1$ integrin in focal adhesions

Glass cover slips were coated with 50 µl of cIV at 2.5 µg/ml, overnight at 29°C. The coated areas were "blocked" for two hours with BSA at 2 mg/ml, in PBS. Human

mesangial cells were processed as before, seeded on each spot of cIV in 50 µl of binding buffer (2500 cells) and allowed to adhere for 5 hours at 37°C. The unbound cells were washed off with PBS. Adherent cells were fixed with 2% paraformaldehyde in HBSS for 30 minutes followed by permeabilization with 0.5% Triton X-100 for 2 minutes. The cells were blocked again with PBS following which 200 µl of hybridoma culture supernatant containing anti-α1 antibody (TS1/7) was added to each spot and incubated at room temperature for 1 hour. The coverslips were then thoroughly washed and rhodamine-conjugated goat anti-mouse antibody (1:100) (Boehringer Mannheim, Indianapolis, IN) was added for one hour. The coverslips were again washed and incubated with anti-vinculin antibodies (Sigma, St. Louis, MO) preconjugated (Quicktag, FITC labeling kit, Boehringer Mannheim, Indianapolis, IN) to FITC labeled goat anti-mouse antibody for 1 hour at room temperature. The coverslips were finally washed, mounted on glass slides and viewed for focal adhesions by co-localization of vinculin with α1 integrin.

Staining of normal human adult kidneys for the presence of β1 integrins

Normal human adult kidney tissue was snap frozen in liquid nitrogen and sections were prepared with a cryostat at 5 µm intervals. The sections were stained using an anti-mouse Vectastain Elite Kit (as described by Wayner et al., 1993) with diamino benzene (DAB) as the chromogen. The following mAbs were used: α1 (TS2/7), α2 (P1H5), α3 (P3D11), α4 (P4G9) and β1 (P5D2). These monoclonal antibodies are available from the following sources and stained the following histological areas as was demonstrated in these studies:

α1 (TS2/7) Martin Hemler, Dana Farber Cancer Center, Boston, MA.
Stained mesangium.

α2 (P1H5) EA Wayner, Fred Hutchinson Cancer Center, Seattle, WA
Stained mesangium.

α3 (P3D11) EA Wayner, Fred Hutchinson Cancer Center, Seattle, WA
Stained the mesangium,
endothelium, visceral and Bowman's
epithelium and capsule.

α4 (P4G9) EA Wayner, Fred Hutchinson Cancer Center, Seattle, WA
Did not stain glomeruli.

β1 (P5D2) EA Wayner, Fred Hutchinson Cancer Center, Seattle, WA
Stained mesangium, endothelium, visceral epithelium, Bowman's epithelium and capsule.

Normal mouse IgG (all isotypes) was used as a negative control.

These studies demonstrated the presence of α1β1 and α2β1 integrin receptors in focal adhesions. Focal adhesions are observed when cells spread in culture on matrix components such as collagen IV, fibronectin or laminin. Integrins cluster at the site of focal adhesions on the cell surface with intracellular fibers such as vinculin staining at these locations within the cell periphery. (see Hynes, et al. *Cell* 69:11-25, 1992 and Burridge, et al. *Ann. Rev. Cell Biol.* 4:487-525, 1988). This supports the hypothesis that mesangial cells use α1β1 and α2β1 integrin receptors to bind to cIV. It has been well established that when a particular integrin receptor is engaged by a specific ligand it can be detected in focal contacts co-localized with certain components of the cytoskeleton such as vinculin. Therefore, we asked whether mesangial cells could localize α1 (or α2 and β1) to focal adhesions when seeded on cIV coated substrates.

α2 or β1 could be detected in focal contacts on cIV regardless of whether mesangial cells were grown in either low or high glucose. Additionally, when mesangial cells were grown in 5 mM glucose and subsequently seeded on cIV coated surfaces, α1 could also be co-localized with vinculin within several focal contacts by dual-label immunofluorescence staining. It is believed that cIV binding in cells maintained in low glucose engages both the α1 and α2 subunits. α1 could be detected in only some of the focal adhesions stained by vinculin. As a control, α1 was not detected in focal contacts when mesangial cells were seeded onto fibronectin coated surfaces regardless of the glucose concentration of the cell culture media.

Immunohistochemical staining of integrin receptor subunits in normal human adult and fetal kidney revealed that both α1 and α2 could be localized within the mesangium. The α1 receptor was diffusely expressed throughout the mesangium whereas the distribution of α2 was more limited and focal. Also consistent with the results we obtained with cultured mesangial cells, β1 and α3 were intensely expressed throughout the mesangium, while α4 could not be detected in either fetal or adult mesangium.

Example 4
Alterations in RNA Production in Human Mesangial Cells
cultured in High and Low Glucose Concentrations

Our efforts have concentrated on finding a way to predict, at early stages after the onset of diabetes, the subjects who will later develop nephropathy. We focused on a major hallmark of diabetic nephropathy, that of mesangial expansion. We first examined mesangial cells in culture, since these cells secrete their surrounding matrix, which is expanded in diabetes; however, biopsied tissue can be treated in the same manner, as will be understood by those skilled in the art. The matrix consists primarily of collagen IV.

Primary cultures of human mesangial cells undergo several phenotypic changes in response to elevated glucose concentrations and glucose-modified ("glycated") collagen IV. These changes included altered cell interactions with the collagen matrix. In elevated glucose concentrations, the α1 subunit underwent a substantial decrease, concomitant with an increase of the α2 integrin subunit. This change was observed with immunoprecipitation and flow cytometry. Further studies with Northern analysis and *in situ* hybridization of the cultured mesangial cells confirmed the integrin reversal. In the studies employing Northern analyses, separate samples of total RNA were isolated from the mesangial cells on each culture plate or alternatively from rat kidneys (see Example 5, below) by a single-step method using RNA STAT-60™ isolation reagent (TEL-TEST "B", INC., Friendswood, TX) according to the manufacturers directions. Briefly, the cells were lysed with RNA STAT-60™ solution by repetitive pipetting; the tissues were cut into small pieces and homogenized in the RNA STAT-60 solution with a high-speed tissue homogenizer (Polytron CH6005, Luzern, Switzerland). The nucleic acid mixture was extracted with 0.2 ml chloroform per 1ml of the RNA STAT-60™ solution. Total RNA was precipitated for 10 min at -80°C in isopropanol, and the pelleted RNA was redissolved in TE buffer. The total RNA was free of DNA and proteins and had a 260/280 wavelength ratio > 1.8.

Northern blot analysis-The RNA samples were denatured in formaldehyde gel-running buffer (20 mM MOPS, 8 mM sodium acetate, mM EDTA, at pH 7.0) containing 6% formaldehyde and 50% formamide by heating at 65°C for 15 min. For each sample 20 mg of RNA was mixed with 6x loading buffer (50% glycerol, 1 mM

EDTA, 0.25% bromphenol blue, 0.25% Xylene cyanol FF), loaded on a 1% agarose gel submerged in 6% formaldehyde running buffer, and run at 3-5 V/cm for 3-4 hours. RNA was transferred from the agarose gel to a nylon membrane (Boehringer Mannheim, Indianapolis, IN) by capillary elution and immobilized by UV cross-linking (Stratalinker UV; Stratagene, La Jolla, CA). The membranes were then incubated in prehybridization solution containing 50% formamide, 5xSSC. 0.02% SDS, 0.1% N-lauroylsarcosine, 2% blocking reagent (Boehringer Mannheim), and 20 mM sodium maleate (pH 7.5) for >3 hours at 42°C. Radiolabeled probes (see Example 5) for the integrin subunits or controls were then added to the prehybridization solution and hybridization was performed overnight at 42°C (for cDNA probe) or 50°C (for antisense RNA probe). After hybridization, the membranes were initially washed in 2x SSC, 0.05% SDS for 10 minutes at room temperature and then washed for an additional 40 minutes at 42°C (for cDNA probe) or 60°C (for antisense RNA probe). Membranes were then exposed to X-ray film (X-Omat RP; Eastman Kodak Co., Rochester, NY) for 1 day at -80°C. After being stripped of previous probes by heating in 0.2x SSC, 0.5% SDS for 10 min at 100°C, the membranes were reprobed as described above. Images of autoradiograms were captured and digitized using a CCD video camera module interfaced with a microcomputer (Macintosh IIcx: Apple Computers Inc., Cupertino, CA) and analyzed using image processing software (NIH Image 1.55b77: public domain).

Cells grown in 25 mM glucose expressed lower levels of α1 integrin than seen in an equivalent amount of RNA from cells grown in 5 mM glucose. Densitometric analysis demonstrated an ≈30% decrease upon averaging the values from four samples. Similar analysis demonstrated ≈30% increase in α2 integrin expression in cells grown in 25 mM glucose.

Example 5
In Situ Hybridization Detecting Expression of Integrins in Kidney Sections Taken at Various Times After Onset of Diabetes

The expression of α1 and α2 integrin receptors was examined in rat kidney sections after the onset of diabetes.

The *in situ* hybridization approach was used to examine kidney sections of streptozotocin-diabetic rats, 2.5 months after induction of diabetes. At this time interval, glomerular changes were still minimal. The streptozotocin-induced diabetic rat model mimics human changes of mesangial expansion and glomerular basement membrane thickening in late nephropathy and is an art accepted model for diabetes and nephropathy.

Female non-pregnant Sprague-Dawley rats were obtained from Brithwood, Minneapolis, MN. The animals weighed 190-210 g at the beginning of the experiments and were given a 52mg/kg intraperitoneal dose of streptozotocin (STZ, Zanazar brand, Upjohn Corp., Kalamazoo, M1) in calcium citrate and calcium carbonate Buffer (pH 4.5) to induce diabetes, while the controls were injected with the same amount of Hanks' balanced salt solution (pH 7.2). The animals were fed on standard rat chow (Purina laboratory chow # 5001. RFG PET@Supply Company, Plymouth, MN), and tap water *ad libitum*. Presence of diabetes was confirmed by detection of >400mg/dl nonfasting plasma glucose levels 10 days post injection by tail vein bleeding using the glucose peroxide method (Beckman glucose analyzer, Beckman Instruments, Inc., Fullerton, CA).

Body weight was determined weekly, blood glucose levels were determined at 4 weeks after induction of diabetes, and on the day before the termination of the experiment, which was 2.5 month from induction of diabetes. Urinary albumin excretion (UAE) was determined by radial immunodiffusion Mancini method, using goat IgG fraction against rat albumin (Cappel Cat. No. 55727) and purified rat albumin (Cappel Cat. No. 55952, Cappel Research Products, Durham, NC), according to previously published procedures (Mauer et al, *Diabetes* 27:959-64, 1978). Rats were sacrificed at 2.5 months after diabetes induction and kidney tissue was perfusionally fixed by injecting freshly prepared 4% paraformaldehyde through the renal artery. This was followed by overnight fixation in 4% paraformaldehyde after removal from the body. The tissue was sectioned at 5 μm and placed on the silane-coated slides (Digene Diagnostics, Inc., Beltsville, MD) for *in situ* hybridization with probes for the α1 and α2 integrin subunits.

2.5 months after injection of STZ, diabetic rats weighted significantly less than controls, whereas their right kidney weight and serum glucose concentration were

significantly increased, as compared to the controls (see Table 1). Diabetic and non-diabetic rats demonstrated no significant difference in glomerular size and albumin excretion at 2.5 month after induction of diabetes (Table 1).

TABLE 1

TISSUE	CONTROL	DIABETIC	S/NS
Body Wt.(g)	390+/-10	200+/-20	S
Right Kidney wt. (g)	1.35+/-0.1	1.8+/-0.1	S
Plasma glucose (mg/dl)	140+/-25	760+/150	S
Glomerular area	1.42+/-0.5	1.45+/-0.6	NS

A 5.4 kb human α2 integrin CDNA clone (Takada, et al., 1989, *supra*) and a rat αl integrin cDNA clone (Ignatius et al, *supra*) in bluescript vector (Stratagene, La Jolla, CA) were used in these experiments. A 1.79 kb α2 integrin cDNA fragment was restriction digested from the EcoRI site. Similarly, a 3.98 kb α1 integrin cDNA fragment was obtained by restriction digestion from the EcoRI site.

cDNA fragments were purified by GENE CLEAN II kit (BIO 101, San Diego, CA) and labeled using the random primer labeling kit (Boehringer Mannheim, Indianapolis, IN) with P^{32}-dCTP (NEN) for Northern blotting and with S^{35}-dCTP (NEN) for *in situ* hybridization. GAPDH and sheep visna virus cDNA (PLV-KS) (Staskus et al, *Virology* 181:228-240, 1991) probes were used as the positive and negative controls respectively. The probes preferably had a specific activity of $2 \times 10^8 - 1 \times 10^9$ dpm/μg.

By Northern blotting, compared to the controls, the diabetic kidneys expressed 113.5% more α1(IV) RNA, 46.5% more α3(IV) RNA, 54.8% less metalloproteinase-2 RNA (MMP-2, an enzyme that cleaves type IV collagen) and 246% more TIMP-1 RNA (a tissue inhibitor of metalloproteinases) with a $p < 0.01$ in all cases as determined by ANOVA.

The expression of αl and α2 integrin RNA was localized using a modification of a previously described method for *in situ* hybridization (Staskus et al. *supra*). 5μm tissue sections on silane-coated slides were fixed in the freshly prepared 4% paraformaldehyde for 10 min. The slides were pretreated with 0.2N HCl for 20 min, 0.15 M Triethanolamine (TEA, Sigma, St. Louis, MO) for 15 min, 0.005% digitonin for 5 min, 3 mg/ml proteinase K (Sigma) for 15 min at 37°C, and O.3% acetic anhydride -

O.1M TEA for 10 min. Hybridizations were performed under stringent hybridization conditions. Stringent hybridization conditions are defined in this specification as 50°C overnight, in 50% formamide, 0.6 M NaCl, 1x Denhardt's solution, 0. 17 mg/ml human COT^{RT} DNA (GIBCO/BRL), 1 mg/ml poly A (Boehringer Mannhieim), 10% (w/v) Dextran sulfate (Sigma), 0. 1 M dithiothreitol (DTT, Boehringer Mannheim), 1 mM EDTA, 0. 1 mM aurinitricarboxylic acid (ATA, Sigma) and S^{35}-dCTP labeled cDNA probe. The next day, the slides were washed in 2x SSC-0.05% SDS for 60 min at 55°C (recipes for SSC and the like can be found in Sambrook, et al., *supra*); further washed in a high stringency washing buffer containing 50% formamide, 0.6 M NaCl, 1 mM EDTA, 5 mM DTT and 10 mM Hepes for 4 days at room temperature. After a brief rinse in 2x SSC, the slides were dehydrated in graded ethanol with 0.3 M ammonium acetate then dipped in Kodak NTB-2 emulsion and exposed for 5 days at 4°C.

After development the slides were stained with hematoxylin-eosin (Surgipath Canada, 1nc., Winnipeg, Canada) and mounted. A ratio of the number of silver grains per cell was used to quantitate the results of *in situ* hybridization. Twenty glomeruli each were counted from each control and diabetic animal. Each glomerulus was assessed for: 1) glomerular area; 2) glomerular perimeter; 3) grains per glomerulus; and 4) number of cell nuclei per glomerulus.

The results were estimated as grains per cell nucleus and grains per glomerular area, as mean +/- SD of 5 animals (20 glomeruli each). (Haase, A.T., [1990]: *In situ* hybridization, CRC Press, 199-217; Nuovo, G.J., [1992] PCR *in situ* hybridization, protocols and applications, Raven Press). Groups were compared with the 2-tailed student t-test. Differences between groups were considered significant at $p<0.05$.

The results are illustrated in Fig. 1. Early after induction of experimental diabetes, the expression of the $\alpha 1$ integrin subunit by glomerular cells was decreased compared to the control, whereas the expression of $\alpha 2$ integrin was increased. The average counts, in diabetic glomeruli hybridized with the $\alpha 1$ integrin probe, were significantly lower than control (Fig. 1). Also, the average counts, in diabetic glomeruli hybridized with the $\alpha 2$ integrin probe, were significantly higher than control (Fig. 1).

Control animals at 2.5 month diabetes expressed on an average a significantly higher level of $\alpha 1$ subunit integrin and significantly lower levels of $\alpha 2$ subunit integrin using unbiased methods of selection of areas for study. The entire section was surveyed

for RNA grains, the regions of the Bowman's space and the background count were excluded by studying a commensurate area of the negative control stained tissue.

Compared to the control, glomerular cells (GC:endothelial, epithelial and mesangial combine) and/or tubular (proximal and distal epithelial) cells (TC) had 36% (GC) less grains for α1 integrin; 86.4% (GC) more grains for α2 integrin; 82(TC)-167% (GC) more grains for α1(IV); 107 (TC)-137% (GC) more grains for α3(IV); 63.6(GC)-65.3%(TC) less MMP-2.

The results of the present study clearly demonstrate that mesangial cells, when cultured in high glucose (25 mM) instead of normal/low glucose (5 mM) alter their RNA production for the integrin subunits α1 and α2. Thus, this phenomenon is observed both at the level of protein and RNA production.

Furthermore, the results of our *in situ* hybridization and immunohistochemical staining experiments show that these changes can be detected in the mesangium of diabetic rat kidney and that human α2 integrin subunit probes and rat α1 integrin subunit probes are functional in both rat and human cells. Work by Mendrick and co-workers (*Lab. Invest.* 72(3):367-375, 1995) has shown that in the rat both integrins α1β1 and α2β1 of mesangial cells interact with collagen; as happens in the human mesangial cells. In the present study, the distribution of α1 and α2 integrin receptor subunit RNA was precisely localized by *in situ* hybridization to the different cell types of the glomerulus and surrounding tubules. Normal rat tissues expressed levels of the α1 subunit and also the α2 subunit RNA, as determined by counting the number ratio of silver grains/cell. However, the streptozotocin-induced diabetic animals had significantly lower levels of RNA for the α1 subunit and significantly higher levels of α2 subunit. A similar distribution of α1 and α2 subunit RNA (silver grains) was seen in the proximal and distal tubular epithelial cells. These data indicate that the distribution of cell surface integrin expression may be regulated by gene expression at the transcriptional level.

In summary, using *in situ* hybridization, similar results were seen in both mesangial cells *in vitro* and in glomeruli from tissue sections probed for the α1 and α2 integrin.

Early after induction of streptozotocin-diabetes in rates, substantial matrix-related gene expression changes occurred. For example, α1 and α2 integrin levels

changes, components of α1β1 and α2β1 integrin cell receptors for tIV (an important component of the renal extracellular matrix) underwent a reversal in levels with less α1 and more α2 integrin being present in glomeruli from kidneys of diabetic rats, when compared to the control. Expression of tIV was increased whereas the expression of MMP-2 which degrades tIV was substantially decreased. TIMP-1, an inhibitor of MMP-2 was increased. The observed matrix changes indicate an imbalance of tIV synthesis and turnover. This dysmetabolism of tIV, apparent in both the glomerular and tubular areas of the kidney, occurred before significant renal functional changes, or matrix accumulation out of proportion to renal enlargement, could be detectable. These changes could have a regulatory role in significant basement membrane thickening and mesangial expansion of diabetic nephropathy.

Collectively, the obtained data indicate that increased glucose concentration induces quantitative changes in receptor synthesis and cell surface integrin expression of human mesangial cells. In the diabetic, all cell systems are exposed to hyperglycemia and it is know that many cell and organ systems are affected by the disease; therefore, other cell types could similarly be used to assess changes in the levels of α1 and/or α2 integrin subunit expression as a measure of a predisposition to a variety of diabetic-induced pathologies. Kyu-Jin, et al. (*supra*) have noted alterations in integrin subunit expression in skin fibroblasts of diabetic patients. This information, in conjunction with the data discussed herein, indicates that altered levels of integrin subunit expression can be detected from a variety of integrin-expressing cells in diabetic nephropathy patients.

These results support the *in vitro* primary human mesangial cell culture data demonstrating that changes in cell surface integrin expression indicate the onset of nephropathic changes.

Example 6
Detection of Altered Levels of α1 and α2 Integrin Subunit Expression in Humans using Blood and Tissue Samples

Patients with insulin-dependent diabetes mellitus (IDDM), individuals at risk for developing IDDM, patients with clinical diabetes nephropathy and healthy age matched volunteers are selected for studies to confirm the presence of altered α1 and α2 integrin subunit expression in integrin-producing cells. Clinical diabetic nephropathy is defined by the presence of persistent proteinuria (urinary AER > 300 μg/day) in sterile urine of

patients with >10 yr duration of disease and concomitant retinopathy and is confirmed by the presence of classic glomerulosclerotic lesions on renal biopsy. Normal, nondiabetic individuals without a family history of hypertension serve as control subjects.

Patients were biopsied as follows: For skin biopsies, a biopsy is taken from the anterior surface of the left forearm by excision under local anaesthetic such as ethyl chloride, see Trevisan, et al. *Diabetes* 41:1239-45, 1992. The biopsy is optionally divided in half. With half of the tissue frozen immediately in liquid nitrogen and the other half placed in Hanks balanced salt solution. The frozen tissue is embedded in paraffin and processed for *in situ* hybridization as has been described above. A portion of the intact tissue is preferably immediately minced and processed for RNA isolation using techniques described above. Remaining minced tissue is gently digested with trypsin to obtain a cell suspension, washed in media containing serum to remove trypsin and plated onto tissue culture dishes containing 10% FCS supplemented DMEM with antibiotics.

Renal biopsies were obtained as follows. Patients should have normal blood pressures, normal coagulation values and platelet counts. Ultrasound was used to precisely localize the kidney. Ultrasound was also used to determine renal size, structural defects and post-void residual urine. Renal biopsies were performed on sedated patients using the Franklin modified Vim-Silverman or Truecut needles available from surgical supply suppliers. The biopsy specimens were immediately examined under a dissecting microscope to ensure that adequate samples of glomeruli were present for subsequent studies to quantitate integrin levels. Biopsied tissue was sectioned and processed for *in situ* hybridization as described in Example 5. In one example, renal samples from diabetic patients who did not show signs of microalbuminuria, but who had diabetic siblings with renal nephropathy were processed for *in situ* hybridization and PCR *in situ* hybridization. Renal samples from diabetic patients without a family history of nephropathy were also studied by PCR *in situ* hybridization to detect altered levels of integrin subunit expression.

PCR *in situ* hybridization is performed as follows. Sections are fixed as described in Example 5 and rinsed in RNase free water. The protocol used is that described by Nuovo, et al. (*Am. J. Surg. Pathol.* 17:683-690, 1993.) Cells are treated

with pepsin and DNase as described. cDNA synthesis is initiated by adding 10µl of a solution containing one or more of the following probes listed in a 5'-3' orientation with their SEQ ID NOS and their nucleic acid location on the respective integrin gene with reverse transcriptase (Perkin-ELmer, Norwalk, Conn.):

α1 integrin primer	SEQ ID NO	NA location
CCAGAGTCACTCTCACAGAG	5	2729-2748
CACAGCGTACACGTACACC	6	1991-2009
CACTTATAGACATCTCCAG	7	646-664

α2 integrin primer	SEQ ID NO	NA location
CATCCATGTTGATGTCTG	8	1733-1750
CATGTGATTCACCGTCAG	9	894-910
GCATATTGAATTGCTCCGAATGTG	10	801-826

The resulting cDNAs are subjected to amplification containing a 1 µM concentration (each) of one or more of the above primers with a paired primer located 5' to the primers provided above. Those skilled in the art will recognize that a variety of other primers could also be used from the α1 and α2 integrin gene sequence to similarly perform PCR *in situ* hybridization. The preferred primers paired with the above primers are provided below.

α1 integrin primer	SEQ ID NO	NA location	SEQ ID Pair
GGCGTATGCACAACGCA	11	2261-2277	5
GCGACAGCTGACCAGTCAGCA	12	1509-1529	6
CACTCCTCCACAGCTCCT	13	251-268	7

α2 integrin primer	SEQ ID NO	NA location	SEQ ID Pair
ACATGTACTCACTGG	14	1593-1608	8
CTCACATGTGGTCCTCTG	15	433-451	9
GTCCTGTTGACCTATCCACTGC	16	296-319	10

The SEQ ID Pair in the above table refers to the paired primer that provides amplification of the sequence positioned between the primer pairs on the respective integrin gene. The PCR products are detected by using an antidigoxigenin-alkaline phosphatase conjugate and the chromagen nitroblue tetrazolium (NBT)-5-bromo-4-chloro-3-indoylphosphate toluidinium (Salt) (BCIP). The counterstain nuclear fast red

is used to stain nuclei. Internal probes located within the nucleic acid regions amplified by PCR can also be used to identify the amplified fragments. Thus, based on the pairings provided above, oligonucleotide probes can be selected between regions 267-645, 1530-1990 and between 2278-2728 for the α1 integrin gene and between regions 320-800, 452-893, 1607-1732 for the α2 integrin gene and hybridized and stained following the *in situ* hybridization methods detailed in Example 5.

A blood sample is also taken from the patient and leukocytes are isolated from blood by centrifugation, followed by hypotonic shock of residual blood cells. The leukocytes are then processed for *in situ* hybridization as has been discussed in the preceding examples.

Results:

PCR *in situ* hybridization with renal tissues demonstrated decreased α1 and increased α2 integrin subunits in the patient with diabetic neuropathy as compared with control tissue.

Quantitative analysis of RNA grains per unit area of kidney glomeruli and tubules was performed by counting silver grains under epi-polarized light.

As shown in Table 2, both glomeruli and tubules of the diabetic neuropathy patient showed significantly decreased α1 integrin levels as compared to the control, whereas α2 integrin levels were significantly increased as compared with control levels.

TABLE 2

	Glomeruli[a]		Tubules[a]	
Sample	α1	α2	α1	α2
Control	156	83	136	101
Diabetic Neuropathy	121[b]	95[c]	89[c]	124[b]

[a] = grains per unit area [b] = $p < 0.05$ [c] = $p < 0.01$

These results confirm the *in vitro* observations in mesangial cells that there is a decrease of the α1 integrin subunit and a concommitant increase of α2 integrin

expression in a diabetic nephropathy. This represents a reversal of mesangial integrins which mediate binding of mesangial cells to collagen IV.

Example 7

Increased Integrin Subunit Expression in Skin Fibroblasts From Diabetic Patients with Nephropathy as Compared with Control Diabetic Patients

Fibroblasts were obtained from skin biopsies from diabetic patients with or without diabetic nephropathy and cultured as described for Example 6. Expression of α3, α5, and beta-1 integrin subunits in the cultured cells was analyzed by Northern blotting and subsequent densitometry, as described above, and using published probes.

For the α3 integrin subunit, the 1.9 SalI fragment described in Takada Y., et al., *J. Cell Biol.* 115:257-266 was used. For the β1 subunit, the 3.6 kb insert of the β1 subunit (the whole cDNA), described in Giancotti and Ruoslahti, *Cell* 60:849-850 (1990) was used. For the α5 subunit, the 3.7 kb SalI-Xba insert of the α5 subunit (the whole cDNA) described in Giancotti and Ruoslahti, *Supra* as used. These probes were radiolabeled and used under the same conditions as those described for Example 6.

The study included five patients per group, five each from the normal, diabetic "slow track" and from the Diabetic "fast track". Both groups of diabetic human subjects had renal function studies and kidney biopsies performed as part of their evaluation as possible candidates for pancreas transplantation. All procedures were approved by the Committee on Human Subjects at the University of Minnesota, and all patients gave written consent. All patients spent one week at the Clinical Research Center (CRC) at the University of Minnesota for pre-pancreas transplant evaluation, during which time they underwent multiple 24-hour urine collections (at least three) for measurements of creatinine clearance and urinary albumin excretion. Blood pressure was measured repeatedly by the CRC nursing staff. HbA1 was used to assess glycemic control. All patients underwent percutaneous kidney biopsy and skin biopsy. Patients were divided into two groups based on criteria of severity of renal lesions determined by morphometric analysis of mesangial functional volume and IDDM duration.

"Normal" samples were kidney biopsies from non-diabetic human subjects, taken to examine for the presence of neoplastic tissue, etc., on which a similar analysis to that performed for the diabetic tissues was done. These subjects underwent similar

renal functional studies to make certain that albuminuria, increased creatinine clearance, or hypertension were not present.

The data, shown below in Table 3, demonstrate a significant increase in α3 and beta-1 subunit expression in the skin fibroblasts of diabetic nephropathy patients as compared with the control diabetic patients.

TABLE 3

Integrin Subunit	Normal Values	Control Diabetic	Nephropathy Diabetics	p
α3	11.5 (9.1-13.3)	10.1 (8.6-12.8)	17.1 (16.1-35.6)	<0.5
α5	36.2 (18.3-46.6)	38.7 (31.6-57.2)	30.3 (13.2-48.4)	
b1	29.9 (24.0-33.4)	24.9 (17.4-30.9)	37.1 (24.2-74.6)	<0.5

While particular embodiments of the invention have been described in detail, it will be apparent to those skilled in the art, that these embodiments are exemplary rather than limiting, and the true scope of the invention is that defined in the following claims.

SEQUENCE LISTING

(1) GENERAL INFORMATION

(i) APPLICANT: Regents of the University of Minnesota

(ii) TITLE OF THE INVENTION: ANALYSIS OF ALPHA INTEGRINS FOR THE DIAGNOSIS OF DIABETIC NEPHROPATHY

(iii) NUMBER OF SEQUENCES: 16

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Merchant & Gould
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90 South 7th Street
(C) CITY: Minneapolis
(D) STATE: MN
(E) COUNTRY: US
(F) ZIP: 55402

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Diskette
(B) COMPUTER: IBM Compatible
(C) OPERATING SYSTEM: DOS
(D) SOFTWARE: FastSEQ Version 1.5

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: Unknown
(B) FILING DATE:
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Kettelberger, Denise
(B) REGISTRATION NUMBER: 33,924
(C) REFERENCE/DOCKET NUMBER: 600.314USWO

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 612-332-5300
(B) TELEFAX: 612-332-9081
(C) TELEX:

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3987 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Genomic DNA
(iii) HYPOTHETICAL: NO
(iv) ANTISENSE: NO
(v) FRAGMENT TYPE:
(vi) ORIGINAL SOURCE:

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 420...3959

(A) NAME/KEY: mat_peptide
(B) LOCATION: 504
(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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AGTATGGAGA GAAGGTCGTT TAAAAAGGCA GATGTCCCTT TAAGGTTTGC TTTGCTGCTG     60
CCCGTGGACT TTAGCCTAAA CAGGGTCCCG CGAAGTTGGC TTTATTTGTC CATGTCTCGG    120
ACACAGCCTG GGTAGCTGCC AGTGAGATTT CAGGGACGGA GCGCGCAAAG GGGGGGGAAA    180
TGTGGCAATC CATCTGGGAT GTGAGACGCG TGGAGAGGGC TTAGCAGCAT TTGACCAAAA    240
CACAGGAAAT CACTCCTCCA CAGCTCCTGG GCGCAGCAGC GGCTGGGGCC ACTGCCGGAC    300
ACCCTCGGAG ACCACACGAG TGACCCAGAG CGCAAGTCGC CAGCGTCCCG GTTCTGCCTG    360
TTCCTGCCAG CTCCTGCCCA CGAACCGGCA CGTAGCTGGT TCCAGCAGCC GCTCCAGCA     419

ATG GTC CCC AGG CGT CCT GCC AGC CTA GAG GTC ACT GTA GCC TGC ATA       467
Met Val Pro Arg Arg Pro Ala Ser Leu Glu Val Thr Val Ala Cys Ile
-28          -25                 -20                 -15

TGG CTT CTC ACG GTC ATC CTA GGC TTC TGC GTC TCC TTC AAT GTT GAT      515
Trp Leu Leu Thr Val Ile Leu Gly Phe Cys Val Ser Phe Asn Val Asp
        -10                 -5                  1

GTG AAA AAC TCA ATG AGT TTC AGT GGC CCA GTA GAG GAC ATG TTT GGA      563
Val Lys Asn Ser Met Ser Phe Ser Gly Pro Val Glu Asp Met Phe Gly
  5                 10                  15                  20

TAC ACT GTT CAA CAA TAT GAA AAC GAA GAA GGC AAA TGG GTT CTT ATT      611
Tyr Thr Val Gln Gln Tyr Glu Asn Glu Glu Gly Lys Trp Val Leu Ile
                25                  30                  35

GGT TCT CCT TTA GTT GGC CAA CCC AAA GCA AGA ACT GGA GAT GTC TAT      659
Gly Ser Pro Leu Val Gly Gln Pro Lys Ala Arg Thr Gly Asp Val Tyr
            40                  45                  50

AAG TGT CCG GTT GGG AGA GAG AGA GCA ATG CCT TGC GTG AAG TTG GAC      707
Lys Cys Pro Val Gly Arg Glu Arg Ala Met Pro Cys Val Lys Leu Asp
        55                  60                  65
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TTG CCA GTT AAC ACA TCG ATC CCC AAT GTC ACA GAA ATA AAG GAA AAC      755
Glu Pro Val Asn Thr Ser Ile Pro Asn Val Thr Glu Ile Lys Glu Asn
     70                  75                  80

ATG ACA TTT GGA TCA ACT TTA GTC ACC AAC CCG AAT GGA GGA TTT CTG      803
Met Thr Phe Gly Ser Thr Leu Val Thr Asn Pro Asn Gly Gly Phe Leu
 85                  90                  95                 100

GCA TGT GGG CCC TTG TAT GCC TAT AGA TGT GGA CAT TTG CAT TAT ACA      851
Ala Cys Gly Pro Leu Tyr Ala Tyr Arg Cys Gly His Leu His Tyr Thr
                105                 110                 115

ACT GGA ATA TGT TCT GAT GTC AGT CCT ACA TTT CAA GTT GTG AAC TCC      899
Thr Gly Ile Cys Ser Asp Val Ser Pro Thr Phe Gln Val Val Asn Ser
            120                 125                 130

TTT GCC CCT GTA CAA GAA TGC AGC ACC CAG CTG GAC ATA GTC ATC GTC      947
Phe Ala Pro Val Gln Glu Cys Ser Thr Gln Leu Asp Ile Val Ile Val
        135                 140                 145

CTG GAT GGC TCC AAC AGC ATC TAC CCC TGG GAA AGT GTC ATC GCC TTT      995
Leu Asp Gly Ser Asn Ser Ile Tyr Pro Trp Glu Ser Val Ile Ala Phe
    150                 155                 160

TTA AAC GAC CTT CTT AAG AGG ATG GAT ATT GGC CCT AAG CAG ACA CAG     1043
Leu Asn Asp Leu Leu Lys Arg Met Asp Ile Gly Pro Lys Gln Thr Gln
165                 170                 175                 180

GTC GGG ATT GTA CAG TAT GGA GAG AAT GTA ACC CAT GAG TTC AAC CTC     1091
Val Gly Ile Val Gln Tyr Gly Glu Asn Val Thr His Glu Phe Asn Leu
                185                 190                 195

AAT AAG TAT TCA TCC ACA GAA GAG GTC CTT GTC GCA GCA AAC AAA ATA     1139
Asn Lys Tyr Ser Ser Thr Glu Glu Val Leu Val Ala Ala Asn Lys Ile
            200                 205                 210

GGC CGA CAG GGA GGC CTC CAA ACG ATG ACA GCC CTT GGA ATA GAC ACA     1187
Gly Arg Gln Gly Gly Leu Gln Thr Met Thr Ala Leu Gly Ile Asp Thr
        215                 220                 225

GCC AGG AAA GAG GCA TTC ACT GAA GCT CGG GGT GCC AGG AGG GGA GTT     1235
Ala Arg Lys Glu Ala Phe Thr Glu Ala Arg Gly Ala Arg Arg Gly Val
    230                 235                 240

AAA AAA GTC ATG GTT ATT GTG ACC GAC GGA GAA TCG CAT GAC AAC TAT     1283
Lys Lys Val Met Val Ile Val Thr Asp Gly Glu Ser His Asp Asn Tyr
245                 250                 255                 260

CGC TGA AAC AGG TCA TCC AAG ACT GCG AGG ACG AAA ACA TTC AGC GAT     1331
Arg Leu Lys Gln Val Ile Gln Asp Cys Glu Asp Glu Asn Ile Gln Arg
                265                 270                 275

TTT TCC ATA GCT ATC CTT GGC CAC TAT AAC AGG GGG AAC TTA AGC ACT     1379
Phe Ser Ile Ala Ile Leu Gly His Tyr Asn Arg Gly Asn Leu Ser Thr
            280                 285                 290
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GAA AAA TTT GTG GAG GAA ATA AAA TCG ATC GCA AGC GAG CCC ACG GAA      1427
Glu Lys Phe Val Glu Glu Ile Lys Ser Ile Ala Ser Glu Pro Thr Glu
        295                 300                 305

AAG CAC TTC TTC AAT GTC TCG GAT GAG TTG GCC CTG GTC ACT ATT GTT      1475
Lys His Phe Phe Asn Val Ser Asp Glu Leu Ala Leu Val Thr Ile Val
    310                 315                 320

AAA GCT CTG GGA GAA AGG ATA TTC GCT TTG GAA GCG ACA GCT GAC CAG      1523
Lys Ala Leu Gly Glu Arg Ile Phe Ala Leu Glu Ala Thr Ala Asp Gln
325                 330                 335                 340

TCA GCA GCT TCA TTT GAG ATG GAA ATG TCT CAG ACT GGC TTC AGT GCT      1571
Ser Ala Ala Ser Phe Glu Met Glu Met Ser Gln Thr Gly Phe Ser Ala
                345                 350                 355

CAC TAC TCC CAG GAC TGG GTC ATG CTT GGA GCG GTG GGA GCC TAT GAC      1619
His Tyr Ser Gln Asp Trp Val Met Leu Gly Ala Val Gly Ala Tyr Asp
            360                 365                 370

TGG AAC GGA ACT GTG GTC ATG CAG AAG GCT AAC CAG ATG GTC ATC CCT      1667
Trp Asn Gly Thr Val Val Met Gln Lys Ala Asn Gln Met Val Ile Pro
        375                 380                 385

CAT AAC ACC ACC TTT CAA ACT GAG CCC GCC AAG ATG AAC GAG CCT CTG      1715
His Asn Thr Thr Phe Gln Thr Glu Pro Ala Lys Met Asn Glu Pro Leu
    390                 395                 400

GCT TCT TAT TTA GGT TAC ACA GTG AAC TCG GCC ACC ATC CCT GGA GAT      1763
Ala Ser Tyr Leu Gly Tyr Thr Val Asn Ser Ala Thr Ile Pro Gly Asp
405                 410                 415                 420

GTG CTC TAC ATC GCT GGG CAG CCT CGG TAC AAT CAT ACG GGC CAG GTC      1811
Val Leu Tyr Ile Ala Gly Gln Pro Arg Tyr Asn His Thr Gly Gln Val
                425                 430                 435

GTC ATC TAC AAG ATG GAG GAT GGG AAC ATC AAC ATT CTG CAG ACA CTC      1859
Val Ile Tyr Lys Met Glu Asp Gly Asn Ile Asn Ile Leu Gln Thr Leu
            440                 445                 450

GGC GGA GAG CAG ATT GGT TCC TAC TTT GGT AGT GTC TTA ACA ACA ATT      1907
Gly Gly Glu Gln Ile Gly Ser Tyr Phe Gly Ser Val Leu Thr Thr Ile
        455                 460                 465

GAC ATC GAC AAA GAT TCT TAT ACT GAT CTG CTT CTC GTC GGG GCC CCC      1955
Asp Ile Asp Lys Asp Ser Tyr Thr Asp Leu Leu Leu Val Gly Ala Pro
    470                 475                 480

ATG TAC ATG GGG ACA GAG AAA GAG GAA CAG GGC AAG GTG TAC GTG TAC      2003
Met Tyr Met Gly Thr Glu Lys Glu Glu Gln Gly Lys Val Tyr Val Tyr
485                 490                 495                 500

GCT GTG AAT CAG ACA AGG TTT GAA TAT CAA ATG AGC CTG GAA CCA ATT      2051
Ala Val Asn Gln Thr Arg Phe Glu Tyr Gln Met Ser Leu Glu Pro Ile
                505                 510                 515
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GGC AGA CCT GCT GCT CAT CCC TGA AGG ATA ATT CAT GCA CGA AAG AAA     2099
Arg Gln Thr Cys Cys Ser Ser Leu Lys Asp Asn Ser Cys Thr Lys Glu
            520                 525                 530

AAC AAG AAT GAG CCC TGC GGG GCC CGC TTC GGA ACA GCA ATT GCT GCT     2147
Asn Lys Asn Glu Pro Cys Gly Ala Arg Phe Gly Thr Ala Ile Ala Ala
        535                 540                 545

GTA AAA GAC CTC AAC GTG GAT GGA TTT AAT GAC GTC GTG ATT GGA GCT     2195
Val Lys Asp Leu Asn Val Asp Gly Phe Asn Asp Val Val Ile Gly Ala
    550                 555                 560

CCG CTG GAA GAT GAC CAC GCA GGA GCT GTG TAC ATT TAT CAT GGC AGT     2243
Pro Leu Glu Asp Asp His Ala Gly Ala Val Tyr Ile Tyr His Gly Ser
565                 570                 575                 580

GGC AAG ACC ATA AGG GAG GCG TAT GCA CAA CGC ATT CCA TCA GGT GGG     2291
Gly Lys Thr Ile Arg Glu Ala Tyr Ala Gln Arg Ile Pro Ser Gly Gly
                585                 590                 595

GAT GGC AAG ACC CTG AAA TTT TTC GGC CAG TCT ATC CAC GGA GAG ATG     2339
Asp Gly Lys Thr Leu Lys Phe Phe Gly Gln Ser Ile His Gly Glu Met
            600                 605                 610

GAT TTA AAT GGT GAC GGT CTG ACT GAC GTG ACC ATT GGA GGC CTT GGT     2387
Asp Leu Asn Gly Asp Gly Leu Thr Asp Val Thr Ile Gly Gly Leu Gly
        615                 620                 625

GGA GCA GCC CTC TTC TGG GCC AGA GAT GTG GCT GTA GTT AAA GTG ACC     2435
Gly Ala Ala Leu Phe Trp Ala Arg Asp Val Ala Val Val Lys Val Thr
    630                 635                 640

ATG AAT TTT GAA CCC AAT AAA GTG AAT ATT CAA AAG AAA AAC TGC CGT     2483
Met Asn Phe Glu Pro Asn Lys Val Asn Ile Gln Lys Lys Asn Cys Arg
645                 650                 655                 660

GTG GAG GGC AAA GAA ACA GTG TGC ATA AAT GCT ACA ATG TGT TTT CAT     2531
Val Glu Gly Lys Glu Thr Val Cys Ile Asn Ala Thr Met Cys Phe His
                665                 670                 675

GTG AAA TTA AAG TCT AAA GAG GAC TCA ATT TAC GAG GCT GAT CTG CAG     2579
Val Lys Leu Lys Ser Lys Glu Asp Ser Ile Tyr Glu Ala Asp Leu Gln
            680                 685                 690

TAC CGT GTC ACC CTT GAT TCA CTG AGG CAG ATA TCA CGG AGC TTT TTT     2627
Tyr Arg Val Thr Leu Asp Ser Leu Arg Gln Ile Ser Arg Ser Phe Phe
        695                 700                 705

TCT GGA ACT CAG GAA AGG AAG ATT CAA AGA AAT ATC ACC GTT CGA GAA     2675
Ser Gly Thr Gln Glu Arg Lys Ile Gln Arg Asn Ile Thr Val Arg Glu
    710                 715                 720

TCA GAA TGC ATC AGG CAC TCC TTC TAC ATG TTG GAC AAA CAT GAC TTT     2723
Ser Glu Cys Ile Arg His Ser Phe Tyr Met Leu Asp Lys His Asp Phe
725                 730                 735                 740
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CAG GAC TCT GTG AGA GTG ACT CTG GAT TTT AAT CTC ACT GAT CCA GAA     2771
Gln Asp Ser Val Arg Val Thr Leu Asp Phe Asn Leu Thr Asp Pro Glu
                745                 750                 755

AAT GGT CCT GTA CTT GAT GAC GCT CTG CCA AAC TCA GTC CAC GAA CAC     2819
Asn Gly Pro Val Leu Asp Asp Ala Leu Pro Asn Ser Val His Glu His
            760                 765                 770

ATT CCC TTT GCC AAA GAC TGT GGA AAC AAG GAA AGA TGC ATT TCA GAC     2867
Ile Pro Phe Ala Lys Asp Cys Gly Asn Lys Glu Arg Cys Ile Ser Asp
        775                 780                 785

CTC ACT CTG AAT GTG TCC ACC ACA GAA AAG AGC CTG CTG ATC GTC AAG     2915
Leu Thr Leu Asn Val Ser Thr Thr Glu Lys Ser Leu Leu Ile Val Lys
    790                 795                 800

TCC CAG CAT GAC AAG TTC AAC GTT AGC CTC ACC GTC AAA AAC AAA GGA     2963
Ser Gln His Asp Lys Phe Asn Val Ser Leu Thr Val Lys Asn Lys Gly
805                 810                 815                 820

GAC AGT GCG TAC AAC ACC AGG ACA GTG GTG CAG CAT TCA CCA AAT CTG     3011
Asp Ser Ala Tyr Asn Thr Arg Thr Val Val Gln His Ser Pro Asn Leu
                825                 830                 835

ATT TTT TCG GGA ATT GAG GAG ATC CAA AAA GAT AGC TGT GAA TCT AAT     3059
Ile Phe Ser Gly Ile Glu Glu Ile Gln Lys Asp Ser Cys Glu Ser Asn
            840                 845                 850

CAA AAT ATC ACT TGC AGA GTT GGA TAT CCT TTC CTA AGA GCA GGA GAA     3107
Gln Asn Ile Thr Cys Arg Val Gly Tyr Pro Phe Leu Arg Ala Gly Glu
        855                 860                 865

ACG GTT ACC TTC AAA ATA ATA TTC CAG TTT AAC ACA TCC CAT CTC TCG     3155
Thr Val Thr Phe Lys Ile Ile Phe Gln Phe Asn Thr Ser His Leu Ser
    870                 875                 880

GAA AAT GCA ATC ATT CAC TTA AGT GCA ACA AGT GAC AGT GAG GAG CCC     3203
Glu Asn Ala Ile Ile His Leu Ser Ala Thr Ser Asp Ser Glu Glu Pro
885                 890                 895                 900

CTG GAA TCT CTT AAT GAT AAT GAA GTA AAT ATT TCC ATC CCA GTA AAA     3251
Leu Glu Ser Leu Asn Asp Asn Glu Val Asn Ile Ser Ile Pro Val Lys
                905                 910                 915

TAT GAA GTT GGA CTG CAG TTT TAC AGT TCT GCG AGT GAA CAT CAC ATT     3299
Tyr Glu Val Gly Leu Gln Phe Tyr Ser Ser Ala Ser Glu His His Ile
            920                 925                 930

TCA GTC GCT GCC AAT GAG ACG ATC CCT GAG TTT ATT AAC TCC ACT GAG     3347
Ser Val Ala Ala Asn Glu Thr Ile Pro Glu Phe Ile Asn Ser Thr Glu
        935                 940                 945

GAC ATT GGG AAT GAA ATT AAT GTC TTC TAT ACG ATT AGA AAG AGG GGG     3395
Asp Ile Gly Asn Glu Ile Asn Val Phe Tyr Thr Ile Arg Lys Arg Gly
    950                 955                 960
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CAT TTC CCA ATG CCA GAA CTT CAG CTG TCA ATT TCA TTC CCC AAT TTG      3443
His Phe Pro Met Pro Glu Leu Gln Leu Ser Ile Ser Phe Pro Asn Leu
965                 970                 975                 980

ACG GCA GAT GGT TAT CCT GTA CTG TAC CCA ATT GGA TGG TCA TCT TCA      3491
Thr Ala Asp Gly Tyr Pro Val Leu Tyr Pro Ile Gly Trp Ser Ser Ser
                985                 990                 995

GAT AAT GTG AAC TGT AGA CCC CGG AGC CTT GAG GAC CCC TTT GGC ATC      3539
Asp Asn Val Asn Cys Arg Pro Arg Ser Leu Glu Asp Pro Phe Gly Ile
            1000                1005                1010

AAC TCT GGG AAG AAA ATG ACA ATA TCG AAG TCT GAG GTT CTC AAA AGA      3587
Asn Ser Gly Lys Lys Met Thr Ile Ser Lys Ser Glu Val Leu Lys Arg
        1015                1020                1025

GGC ACA ATC CAG GAC TGC AGT AGT ACG TGT GGA GTT GCC ACC ATC ACG      3635
Gly Thr Ile Gln Asp Cys Ser Ser Thr Cys Gly Val Ala Thr Ile Thr
    1030                1035                1040

TGT AGC CTC CTT CCT TCC GAC CTG AGT CAA GTG AAT GTC TCG CTC CTC      3683
Cys Ser Leu Leu Pro Ser Asp Leu Ser Gln Val Asn Val Ser Leu Leu
1045                1050                1055                1060

CTG TGG AAA CCG ACT TTC ATA AGA GCA CAT TTT TCC AGC TTA AAC CTT      3731
Leu Trp Lys Pro Thr Phe Ile Arg Ala His Phe Ser Ser Leu Asn Leu
                1065                1070                1075

ACT CTA AGA GGA GAA CTT AAG AGT GAA AAT TCA TCG CTG ACT TTA AGT      3779
Thr Leu Arg Gly Glu Leu Lys Ser Glu Asn Ser Ser Leu Thr Leu Ser
            1080                1085                1090

AGC AGC AAC CGG AAG CGA GAG CTG GCT ATT CAG ATA TCC AAA GAC GGG      3827
Ser Ser Asn Arg Lys Arg Glu Leu Ala Ile Gln Ile Ser Lys Asp Gly
        1095                1100                1105

CTC CCA GGC AGA GTG CCG CTG TGG GTT ATC CTC CTG AGC GCC TTC GCG      3875
Leu Pro Gly Arg Val Pro Leu Trp Val Ile Leu Leu Ser Ala Phe Ala
    1110                1115                1120

GGG CTA CTG CTG CTA ATG CTC CTT ATA TTG GCT CTG TGG AAG ATT GGA      3923
Gly Leu Leu Leu Leu Met Leu Leu Ile Leu Ala Leu Trp Lys Ile Gly
1125                1130                1135                1140

TTC TTC AAA AGG CCA CTG AAG AAG AAA ATG GAG AAA TGAAAGGTTT          3969
Phe Phe Lys Arg Pro Leu Lys Lys Lys Met Glu Lys
                1145                1150

CATAGAAAAA AAAAAAAAAA                                              3987
```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1180 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein
(iii) HYPOTHETICAL: NO
(iv) ANTISENSE: NO
(v) FRAGMENT TYPE: N-terminal
(vi) ORIGINAL SOURCE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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Met Val Pro Arg Arg Pro Ala Ser Leu Glu Val Thr Val Ala Cys Ile
 -28         -25                 -20                 -15

Trp Leu Leu Thr Val Ile Leu Gly Phe Cys Val Ser Phe Asn Val Asp
        -10                 -5                  1

Val Lys Asn Ser Met Ser Phe Ser Gly Pro Val Glu Asp Met Phe Gly
  5                  10                  15                  20

Tyr Thr Val Gln Gln Tyr Glu Asn Glu Glu Gly Lys Trp Val Leu Ile
                25                  30                  35

Gly Ser Pro Leu Val Gly Gln Pro Lys Ala Arg Thr Gly Asp Val Tyr
            40                  45                  50

Lys Cys Pro Val Gly Arg Glu Arg Ala Met Pro Cys Val Lys Leu Asp
         55                  60                  65

Glu Pro Val Asn Thr Ser Ile Pro Asn Val Thr Glu Ile Lys Glu Asn
     70                  75                  80

Met Thr Phe Gly Ser Thr Leu Val Thr Asn Pro Asn Gly Gly Phe Leu
 85                  90                  95                  100

Ala Cys Gly Pro Leu Tyr Ala Tyr Arg Cys Gly His Leu His Tyr Thr
                105                 110                 115

Thr Gly Ile Cys Ser Asp Val Ser Pro Thr Phe Gln Val Val Asn Ser
            120                 125                 130

Phe Ala Pro Val Gln Glu Cys Ser Thr Gln Leu Asp Ile Val Ile Val
        135                 140                 145

Leu Asp Gly Ser Asn Ser Ile Tyr Pro Trp Glu Ser Val Ile Ala Phe
    150                 155                 160

Leu Asn Asp Leu Leu Lys Arg Met Asp Ile Gly Pro Lys Gln Thr Gln
165                 170                 175                 180

Val Gly Ile Val Gln Tyr Gly Glu Asn Val Thr His Glu Phe Asn Leu
                185                 190                 195
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Asn Lys Tyr Ser Ser Thr Glu Glu Val Leu Val Ala Ala Asn Lys Ile
            200                 205                 210

Gly Arg Gln Gly Gly Leu Gln Thr Met Thr Ala Leu Gly Ile Asp Thr
        215                 220                 225

Ala Arg Lys Glu Ala Phe Thr Glu Ala Arg Gly Ala Arg Arg Gly Val
    230                 235                 240

Lys Lys Val Met Val Ile Val Thr Asp Gly Glu Ser His Asp Asn Tyr
245                 250                 255                 260

Arg Leu Lys Gln Val Ile Gln Asp Cys Glu Asp Glu Asn Ile Gln Arg
                265                 270                 275

Phe Ser Ile Ala Ile Leu Gly His Tyr Asn Arg Gly Asn Leu Ser Thr
            280                 285                 290

Glu Lys Phe Val Glu Glu Ile Lys Ser Ile Ala Ser Glu Pro Thr Glu
        295                 300                 305

Lys His Phe Phe Asn Val Ser Asp Glu Leu Ala Leu Val Thr Ile Val
    310                 315                 320

Lys Ala Leu Gly Glu Arg Ile Phe Ala Leu Glu Ala Thr Ala Asp Gln
325                 330                 335                 340

Ser Ala Ala Ser Phe Glu Met Glu Met Ser Gln Thr Gly Phe Ser Ala
                345                 350                 355

His Tyr Ser Gln Asp Trp Val Met Leu Gly Ala Val Gly Ala Tyr Asp
            360                 365                 370

Trp Asn Gly Thr Val Val Met Gln Lys Ala Asn Gln Met Val Ile Pro
        375                 380                 385

His Asn Thr Thr Phe Gln Thr Glu Pro Ala Lys Met Asn Glu Pro Leu
    390                 395                 400

Ala Ser Tyr Leu Gly Tyr Thr Val Asn Ser Ala Thr Ile Pro Gly Asp
405                 410                 415                 420

Val Leu Tyr Ile Ala Gly Gln Pro Arg Tyr Asn His Thr Gly Gln Val
                425                 430                 435

Val Ile Tyr Lys Met Glu Asp Gly Asn Ile Asn Ile Leu Gln Thr Leu
            440                 445                 450

Gly Gly Glu Gln Ile Gly Ser Tyr Phe Gly Ser Val Leu Thr Thr Ile
        455                 460                 465

Asp Ile Asp Lys Asp Ser Tyr Thr Asp Leu Leu Leu Val Gly Ala Pro
    470                 475                 480

Met Tyr Met Gly Thr Glu Lys Glu Glu Gln Gly Lys Val Tyr Val Tyr
485                 490                 495                 500
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Ala Val Asn Gln Thr Arg Phe Glu Tyr Gln Met Ser Leu Glu Pro Ile
505 510 515

Arg Gln Thr Cys Cys Ser Ser Leu Lys Asp Asn Ser Cys Thr Lys Glu
520 525 530

Asn Lys Asn Glu Pro Cys Gly Ala Arg Phe Gly Thr Ala Ile Ala Ala
535 540 545

Val Lys Asp Leu Asn Val Asp Gly Phe Asn Asp Val Val Ile Gly Ala
550 555 560

Pro Leu Glu Asp Asp His Ala Gly Ala Val Tyr Ile Tyr His Gly Ser
565 570 575 580

Gly Lys Thr Ile Arg Glu Ala Tyr Ala Gln Arg Ile Pro Ser Gly Gly
585 590 595

Asp Gly Lys Thr Leu Lys Phe Phe Gly Gln Ser Ile His Gly Glu Met
600 605 610

Asp Leu Asn Gly Asp Gly Leu Thr Asp Val Thr Ile Gly Gly Leu Gly
615 620 625

Gly Ala Ala Leu Phe Trp Ala Arg Asp Val Ala Val Val Lys Val Thr
630 635 640

Met Asn Phe Glu Pro Asn Lys Val Asn Ile Gln Lys Lys Asn Cys Arg
645 650 655 660

Val Glu Gly Lys Glu Thr Val Cys Ile Asn Ala Thr Met Cys Phe His
665 670 675

Val Lys Leu Lys Ser Lys Glu Asp Ser Ile Tyr Glu Ala Asp Leu Gln
680 685 690

Tyr Arg Val Thr Leu Asp Ser Leu Arg Gln Ile Ser Arg Ser Phe Phe
695 700 705

Ser Gly Thr Gln Glu Arg Lys Ile Gln Arg Asn Ile Thr Val Arg Glu
710 715 720

Ser Glu Cys Ile Arg His Ser Phe Tyr Met Leu Asp Lys His Asp Phe
725 730 735 740

Gln Asp Ser Val Arg Val Thr Leu Asp Phe Asn Leu Thr Asp Pro Glu
745 750 755

Asn Gly Pro Val Leu Asp Asp Ala Leu Pro Asn Ser Val His Glu His
760 765 770

Ile Pro Phe Ala Lys Asp Cys Gly Asn Lys Glu Arg Cys Ile Ser Asp
775 780 785

Leu Thr Leu Asn Val Ser Thr Thr Glu Lys Ser Leu Leu Ile Val Lys
790 795 800

Ser Gln His Asp Lys Phe Asn Val Ser Leu Thr Val Lys Asn Lys Gly
805 810 815 820

Asp Ser Ala Tyr Asn Thr Arg Thr Val Val Gln His Ser Pro Asn Leu
825 830 835

Ile Phe Ser Gly Ile Glu Glu Ile Gln Lys Asp Ser Cys Glu Ser Asn
840 845 850

Gln Asn Ile Thr Cys Arg Val Gly Tyr Pro Phe Leu Arg Ala Gly Glu
855 860 865

Thr Val Thr Phe Lys Ile Ile Phe Gln Phe Asn Thr Ser His Leu Ser
870 875 880

Glu Asn Ala Ile Ile His Leu Ser Ala Thr Ser Asp Ser Glu Glu Pro
885 890 895 900

Leu Glu Ser Leu Asn Asp Asn Glu Val Asn Ile Ser Ile Pro Val Lys
905 910 915

Tyr Glu Val Gly Leu Gln Phe Tyr Ser Ser Ala Ser Glu His His Ile
920 925 930

Ser Val Ala Ala Asn Glu Thr Ile Pro Glu Phe Ile Asn Ser Thr Glu
935 940 945

Asp Ile Gly Asn Glu Ile Asn Val Phe Tyr Thr Ile Arg Lys Arg Gly
950 955 960

His Phe Pro Met Pro Glu Leu Gln Leu Ser Ile Ser Phe Pro Asn Leu
965 970 975 980

Thr Ala Asp Gly Tyr Pro Val Leu Tyr Pro Ile Gly Trp Ser Ser Ser
985 990 995

Asp Asn Val Asn Cys Arg Pro Arg Ser Leu Glu Asp Pro Phe Gly Ile
1000 1005 1010

Asn Ser Gly Lys Lys Met Thr Ile Ser Lys Ser Glu Val Leu Lys Arg
1015 1020 1025

Gly Thr Ile Gln Asp Cys Ser Ser Thr Cys Gly Val Ala Thr Ile Thr
1030 1035 1040

Cys Ser Leu Leu Pro Ser Asp Leu Ser Gln Val Asn Val Ser Leu Leu
1045 1050 1055 1060

Leu Trp Lys Pro Thr Phe Ile Arg Ala His Phe Ser Ser Leu Asn Leu
1065 1070 1075

Thr Leu Arg Gly Glu Leu Lys Ser Glu Asn Ser Ser Leu Thr Leu Ser
1080 1085 1090

Ser Ser Asn Arg Lys Arg Glu Leu Ala Ile Gln Ile Ser Lys Asp Gly
1095 1100 1105

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Leu Pro Gly Arg Val Pro Leu Trp Val Ile Leu Leu Ser Ala Phe Ala
    1110                1115                1120

Gly Leu Leu Leu Leu Met Leu Leu Ile Leu Ala Leu Trp Lys Ile Gly
1125                1130                1135                1140

Phe Phe Lys Arg Pro Leu Lys Lys Lys Met Glu Lys
                1145                1150
```

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5373 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA
(iii) HYPOTHETICAL: NO
(iv) ANTISENSE: NO
(v) FRAGMENT TYPE:
(vi) ORIGINAL SOURCE:
(ix) FEATURE:

(A) NAME/KEY: Coding Sequence
(B) LOCATION: 49...3591
(D) OTHER INFORMATION:

(A) NAME/KEY: mat_peptide
(B) LOCATION: 136
(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

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GAATTCCTGC AAACCCAGCG CAACTACGGT CCCCCGGTCA GACCCAGG ATG GGG CCA      57
                                                      Met Gly Pro
                                                      -29

GAA CGG ACA GGG GCC GCG CCG CTG CCG CTG CTG CTG GTG TTA GCG CTC      105
Glu Arg Thr Gly Ala Ala Pro Leu Pro Leu Leu Leu Val Leu Ala Leu
    -25                 -20                 -15

AGT CAA GGC ATT TTA AAT TGT TGT TTG GCC TAC AAT GTT GGT CTC CCA      153
Ser Gln Gly Ile Leu Asn Cys Cys Leu Ala Tyr Asn Val Gly Leu Pro
-10                 -5                  1                   5

GAA GCA AAA ATA TTT TCC GGT CCT TCA AGT GAA CAG TTT GGG TAT GCA      201
Glu Ala Lys Ile Phe Ser Gly Pro Ser Ser Glu Gln Phe Gly Tyr Ala
            10                  15                  20

GTG CAG CAG TTT ATA AAT CCA AAA GGC AAC TGG TTA CTG GTT GGT TCA      249
Val Gln Gln Phe Ile Asn Pro Lys Gly Asn Trp Leu Leu Val Gly Ser
        25                  30                  35
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CCC TGG AGT GGC TTT CCT GAG AAC CGA ATG GGA GAT GTG TAT AAA TGT      297
Pro Trp Ser Gly Phe Pro Glu Asn Arg Met Gly Asp Val Tyr Lys Cys
     40                  45                  50

CCT GTT GAC CTA TCC ACT GCC ACA TGT GAA AAA CTA AAT TTG CAA ACT      345
Pro Val Asp Leu Ser Thr Ala Thr Cys Glu Lys Leu Asn Leu Gln Thr
 55                  60                  65                  70

TCA ACA AGC ATT CCA AAT GTT ACT GAG ATG AAA ACC AAC ATG AGC CTC      393
Ser Thr Ser Ile Pro Asn Val Thr Glu Met Lys Thr Asn Met Ser Leu
                 75                  80                  85

GGC TTG ATC CTC ACC AGG AAC ATG GGA ACT GGA GGT TTT CTC ACA TGT      441
Gly Leu Ile Leu Thr Arg Asn Met Gly Thr Gly Gly Phe Leu Thr Cys
            90                  95                 100

GGT CCT CTG TGG GCA CAG CAA TGT GGG AAT CAG TAT TAC ACA ACG GGT      489
Gly Pro Leu Trp Ala Gln Gln Cys Gly Asn Gln Tyr Tyr Thr Thr Gly
        105                 110                 115

GTG TGT TCT GAC ATC AGT CCT GAT TTT CAG CTC TCA GCC AGC TTC TCA      537
Val Cys Ser Asp Ile Ser Pro Asp Phe Gln Leu Ser Ala Ser Phe Ser
    120                 125                 130

CCT GCA ACT CAG CCC TGC CCT TCC CTC ATA GAT GTT GTG GTT GTG TGT      585
Pro Ala Thr Gln Pro Cys Pro Ser Leu Ile Asp Val Val Val Val Cys
135                 140                 145                 150

GAT GAA TCA AAT AGT ATT TAT CCT TGG GAT GCA GTA AAG AAT TTT TTG      633
Asp Glu Ser Asn Ser Ile Tyr Pro Trp Asp Ala Val Lys Asn Phe Leu
                155                 160                 165

GAA AAA TTT GTA CAA GGC CTT GAT ATA GGC CCC ACA AAG ACA CAG GTG      681
Glu Lys Phe Val Gln Gly Leu Asp Ile Gly Pro Thr Lys Thr Gln Val
            170                 175                 180

GGG TTA ATT CAG TAT GCC AAT AAT CCA AGA GTT GTG TTT AAC TTG AAC      729
Gly Leu Ile Gln Tyr Ala Asn Asn Pro Arg Val Val Phe Asn Leu Asn
        185                 190                 195

ACA TAT AAA ACC AAA GAA GAA ATG ATT GTA GCA ACA TCC CAG ACA TCC      777
Thr Tyr Lys Thr Lys Glu Glu Met Ile Val Ala Thr Ser Gln Thr Ser
    200                 205                 210

CAA TAT GGT GGG GAC CTC ACA AAC ACA TTC GGA GCA ATT CAA TAT GCA      825
Gln Tyr Gly Gly Asp Leu Thr Asn Thr Phe Gly Ala Ile Gln Tyr Ala
215                 220                 225                 230

AGA AAA TAT GCC TAT TCA GCA GCT TCT GGT GGG CGA CGA AGT GCT ACG      873
Arg Lys Tyr Ala Tyr Ser Ala Ala Ser Gly Gly Arg Arg Ser Ala Thr
                235                 240                 245

AAA GTA ATG GTA GTT GTA ACT GAC GGT GAA TCA CAT GAT GGT TCA ATG      921
Lys Val Met Val Val Val Thr Asp Gly Glu Ser His Asp Gly Ser Met
            250                 255                 260
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TTG AAA GCT GTG ATT GAT CAA TGC AAC CAT GAC AAT ATA CTG AGG TTT      969
Leu Lys Ala Val Ile Asp Gln Cys Asn His Asp Asn Ile Leu Arg Phe
        265                 270                 275

GGC ATA GCA GTT CTT GGG TAC TTA AAC AGA AAC GCC CTT GAT ACT AAA     1017
Gly Ile Ala Val Leu Gly Tyr Leu Asn Arg Asn Ala Leu Asp Thr Lys
    280                 285                 290

AAT TTA ATA AAA GAA ATA AAA GCG ATC GCT AGT ATT CCA ACA GAA AGA     1065
Asn Leu Ile Lys Glu Ile Lys Ala Ile Ala Ser Ile Pro Thr Glu Arg
295                 300                 305                 310

TAC TTT TTC AAT GTG TCT GAT GAA GCA GCT CTA CTA GAA AAG GCT GGG     1113
Tyr Phe Phe Asn Val Ser Asp Glu Ala Ala Leu Leu Glu Lys Ala Gly
                315                 320                 325

ACA TTA GGA GAA CAA ATT TTC AGC ATT GAA GGT ACT GTT CAA GGA GGA     1161
Thr Leu Gly Glu Gln Ile Phe Ser Ile Glu Gly Thr Val Gln Gly Gly
            330                 335                 340

GAC AAC TTT CAG ATG GAA ATG TCA CAA GTG GGA TTC AGT GCA GAT TAC     1209
Asp Asn Phe Gln Met Glu Met Ser Gln Val Gly Phe Ser Ala Asp Tyr
        345                 350                 355

TCT TCT CAA AAT GAT ATT CTG ATG CTG GGT GCA GTG GGA GCT TTT GGC     1257
Ser Ser Gln Asn Asp Ile Leu Met Leu Gly Ala Val Gly Ala Phe Gly
    360                 365                 370

TGG AGT GGG ACC ATT GTC CAG AAG ACA TCT CAT GGC CAT TTG ATC TTT     1305
Trp Ser Gly Thr Ile Val Gln Lys Thr Ser His Gly His Leu Ile Phe
375                 380                 385                 390

CCT AAA CAA GCC TTT GAC CAA ATT CTG CAG GAC AGA AAT CAC AGT TCA     1353
Pro Lys Gln Ala Phe Asp Gln Ile Leu Gln Asp Arg Asn His Ser Ser
                395                 400                 405

TAT TTA GGT TAC TCT GTG GCT GCA ATT TCT ACT GGA GAA AGC ACT CAC     1401
Tyr Leu Gly Tyr Ser Val Ala Ala Ile Ser Thr Gly Glu Ser Thr His
            410                 415                 420

TTT GTT GCT GGT GCT CCT CGG GCA AAT TAT ACC GGC CAG ATA GTG CTA     1449
Phe Val Ala Gly Ala Pro Arg Ala Asn Tyr Thr Gly Gln Ile Val Leu
        425                 430                 435

TAT AGT GTG AAT GAG AAT GGC AAT ATC ACG GTT ATT CAG GCT CAC CGA     1497
Tyr Ser Val Asn Glu Asn Gly Asn Ile Thr Val Ile Gln Ala His Arg
    440                 445                 450

GGT GAC CAG ATT GGC TCC TAT TTT GGT AGT GTG CTG TGT TCA GTT GAT     1545
Gly Asp Gln Ile Gly Ser Tyr Phe Gly Ser Val Leu Cys Ser Val Asp
455                 460                 465                 470

GTG GAT AAA GAC ACC ATT ACA GAC GTG CTC TTG GTA GGT GCA CCA ATG     1593
Val Asp Lys Asp Thr Ile Thr Asp Val Leu Leu Val Gly Ala Pro Met
                475                 480                 485
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TAC ATG AGT GAC CTA AAG AAA GAG GAA GGA AGA GTC TAC CTG TTT ACT    1641
Tyr Met Ser Asp Leu Lys Lys Glu Glu Gly Arg Val Tyr Leu Phe Thr
            490                 495                 500

ATC AAA AAG GGC ATT TTG GGT CAG CAC CAA TTT CTT GAA GGC CCC GAG    1689
Ile Lys Lys Gly Ile Leu Gly Gln His Gln Phe Leu Glu Gly Pro Glu
        505                 510                 515

GGC ATT GAA AAC ACT CGA TTT GGT TCA GCA ATT GCA GCT CTT TCA GAC    1737
Gly Ile Glu Asn Thr Arg Phe Gly Ser Ala Ile Ala Ala Leu Ser Asp
    520                 525                 530

ATC AAC ATG GAT GGC TTT AAT GAT GTG ATT GTT GGT TCA CCA CTA GAA    1785
Ile Asn Met Asp Gly Phe Asn Asp Val Ile Val Gly Ser Pro Leu Glu
535                 540                 545                 550

AAT CAG AAT TCT GGA GCT GTA TAC ATT TAC AAT GGT CAT CAG GGC ACT    1833
Asn Gln Asn Ser Gly Ala Val Tyr Ile Tyr Asn Gly His Gln Gly Thr
                555                 560                 565

ATC CGC ACA AAG TAT TCC CAG AAA ATC TTG GGA TCC GAT GGA GCC TTT    1881
Ile Arg Thr Lys Tyr Ser Gln Lys Ile Leu Gly Ser Asp Gly Ala Phe
            570                 576                 580

AGG AGC CAT CTC CAG TAC TTT GGG AGG TCC TTG GAT GGC TAT GGA GAT    1929
Arg Ser His Leu Gln Tyr Phe Gly Arg Ser Leu Asp Gly Tyr Gly Asp
        585                 590                 595

TTA AAT GGG GAT TCC ATC ACC GAT GTG TCT ATT GGT GCC TTT GGA CAA    1977
Leu Asn Gly Asp Ser Ile Thr Asp Val Ser Ile Gly Ala Phe Gly Gln
    600                 605                 610

GTG GTT CAA CTC TGG TCA CAA AGT ATT GCT GAT GTA GCT ATA GAA GCT    2025
Val Val Gln Leu Trp Ser Gln Ser Ile Ala Asp Val Ala Ile Glu Ala
615                 620                 625                 630

TCA TTC ACA CCA GAA AAA ATC ACT TTG GTC AAC AAG AAT GCT CAG ATA    2073
Ser Phe Thr Pro Glu Lys Ile Thr Leu Val Asn Lys Asn Ala Gln Ile
                635                 640                 645

ATT CTC AAA CTC TGC TTC AGT GCA AAG TTC AGA CCT ACT AAG CAA AAC    2121
Ile Leu Lys Leu Cys Phe Ser Ala Lys Phe Arg Pro Thr Lys Gln Asn
            650                 655                 660

AAT CAA GTG GCC ATT GTA TAT AAC ATC ACA CTT GAT GCA GAT GGA TTT    2169
Asn Gln Val Ala Ile Val Tyr Asn Ile Thr Leu Asp Ala Asp Gly Phe
        665                 670                 675

TCA TCC AGA GTA ACC TCC AGG GGG TTA TTT AAA GAA AAC AAT GAA AGG    2217
Ser Ser Arg Val Thr Ser Arg Gly Leu Phe Lys Glu Asn Asn Glu Arg
    680                 685                 690

TGC CTG CAG AAG AAT ATG GTA GTA AAT CAA GCA CAG AGT TGC CCC GAG    2265
Cys Leu Gln Lys Asn Met Val Val Asn Gln Ala Gln Ser Cys Pro Glu
695                 700                 715                 720
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CAC ATC ATT TAT ATA CAG GAG CCC TCT GAT GTT GTC AAC TCT TTG GAT    2313
His Ile Ile Tyr Ile Gln Glu Pro Ser Asp Val Val Asn Ser Leu Asp
                725                 730                 735

TTG CGT GTG GAC ATC AGT CTG GAA AAC CCT GGC ACT AGC CCT GCC CTT    2361
Leu Arg Val Asp Ile Ser Leu Glu Asn Pro Gly Thr Ser Pro Ala Leu
            740                 745                 750

GAA GCC TAT TCT GAG ACT GCC AAG GTC TTC AGT ATT CCT TTC CAC AAA    2409
Glu Ala Tyr Ser Glu Thr Ala Lys Val Phe Ser Ile Pro Phe His Lys
        755                 760                 765

GAC TGT GGT GAG GAT GGA CTT TGC ATT TCT GAT CTA GTC CTA GAT GTC    2457
Asp Cys Gly Glu Asp Gly Leu Cys Ile Ser Asp Leu Val Leu Asp Val
    760                 765                 770

CGA CAA ATA CCA GCT GCT CAA GAA CAA CCC TTT ATT GTC AGC AAC CAA    2505
Arg Gln Ile Pro Ala Ala Gln Glu Gln Pro Phe Ile Val Ser Asn Gln
775                 780                 785                 790

AAC AAA AGG TTA ACA TTT TCA GTA ACA CTG AAA AAT AAA AGG GAA AGT    2553
Asn Lys Arg Leu Thr Phe Ser Val Thr Leu Lys Asn Lys Arg Glu Ser
                795                 800                 805

GCA TAC AAC ACT GGA ATT GTT GTT GAT TTT TCA GAA AAC TTG TTT TTT    2601
Ala Tyr Asn Thr Gly Ile Val Val Asp Phe Ser Glu Asn Leu Phe Phe
            810                 815                 820

GCA TCA TTC TCC CTA CCG GTT GAT GGG ACA GAA GTA ACA TGC CAG GTG    2649
Ala Ser Phe Ser Leu Pro Val Asp Gly Thr Glu Val Thr Cys Gln Val
        825                 830                 835

GCT GCA TCT CAG AAG TCT GTT GCC TGC GAT GTA GGC TAC CCT GCT TTA    2697
Ala Ala Ser Gln Lys Ser Val Ala Cys Asp Val Gly Tyr Pro Ala Leu
    840                 845                 850

AAG AGA GAA CAA CAG GTG ACT TTT ACT ATT AAC TTT GAC TTC AAT CTT    2745
Lys Arg Glu Gln Gln Val Thr Phe Thr Ile Asn Phe Asp Phe Asn Leu
855                 860                 865                 870

CAA AAC CTT CAG AAT CAG GCG TCT CTC AGT TTC CAA GCC TTA AGT GAA    2793
Gln Asn Leu Gln Asn Gln Ala Ser Leu Ser Phe Gln Ala Leu Ser Glu
                875                 880                 885

AGC CAA GAA GAA AAC AAG GCT GAT AAT TTG GTC AAC CTC AAA ATT CCT    2841
Ser Gln Glu Glu Asn Lys Ala Asp Asn Leu Val Asn Leu Lys Ile Pro
            890                 895                 900

CTC CTG TAT GAT GCT GAA ATT CAC TTA ACA AGA TCT ACC AAC ATA AAT    2889
Leu Leu Tyr Asp Ala Glu Ile His Leu Thr Arg Ser Thr Asn Ile Asn
        905                 910                 915

TTT TAT GAA ATC TCT TCG GAT GGG AAT GTT CCT TCA ATC GTG CAC AGT    2937
Phe Tyr Glu Ile Ser Ser Asp Gly Asn Val Pro Ser Ile Val His Ser
    920                 925                 930
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TTT GAA GAT GTT GGT CCA AAA TTC ATC TTC TCC CTG AAG GTA ACA ACA     2985
Phe Glu Asp Val Gly Pro Lys Phe Ile Phe Ser Leu Lys Val Thr Thr
935                 940                 945                 950

GGA AGT GTT CCA GTA AGC ATG GCA ACT GTA ATC ATC CAC ATC CCT CAG     3033
Gly Ser Val Pro Val Ser Met Ala Thr Val Ile Ile His Ile Pro Gln
                955                 960                 965

TAT ACC AAA GAA AAG AAC CCA CTG ATG TAC CTA ACT GGG GTG CAA ACA     3081
Tyr Thr Lys Glu Lys Asn Pro Leu Met Tyr Leu Thr Gly Val Gln Thr
            970                 975                 980

GAC AAG GCT GGT GAC ATC AGT TGT AAT GCA GAT ATC AAT CCA CTG AAA     3129
Asp Lys Ala Gly Asp Ile Ser Cys Asn Ala Asp Ile Asn Pro Leu Lys
        985                 990                 995

ATA GGA CAA ACA TCT TCT TCT GTA TCT TTC AAA AGT GAA AAT TTC AGG     3177
Ile Gly Gln Thr Ser Ser Ser Val Ser Phe Lys Ser Glu Asn Phe Arg
    1000                1005                1010

CAC ACC AAA GAA TTG AAC TGC AGA ACT GCT TCC TGT AGT AAT GTT ACC     3225
His Thr Lys Glu Leu Asn Cys Arg Thr Ala Ser Cys Ser Asn Val Thr
1015                1020                1025                1030

TGC TGG TTG AAA GAC GTT CAC ATG AAA GGA GAA TAC TTT GTT AAT GTG     3273
Cys Trp Leu Lys Asp Val His Met Lys Gly Glu Tyr Phe Val Asn Val
                1035                1040                1045

ACT ACC AGA ATT TGG AAC GGG ACT TTC GCA TCA TCA ACG TTC CAG ACA     3321
Thr Thr Arg Ile Trp Asn Gly Thr Phe Ala Ser Ser Thr Phe Gln Thr
            1050                1055                1060

GTA CAG CTA ACG GCA GCT GCA GAA ATC AAC ACC TAT AAC CCT GAG ATA     3369
Val Gln Leu Thr Ala Ala Ala Glu Ile Asn Thr Tyr Asn Pro Glu Ile
        1065                1070                1075

TAT GTG ATT GAA GAT AAC ACT GTT ACG ATT CCC CTG ATG ATA ATG AAA     3417
Tyr Val Ile Glu Asp Asn Thr Val Thr Ile Pro Leu Met Ile Met Lys
    1080                1085                1090

CCT GAT GAG AAA GCC GAA GTA CCA ACA GGA GTT ATA ATA GGA AGT ATA     3465
Pro Asp Glu Lys Ala Glu Val Pro Thr Gly Val Ile Ile Gly Ser Ile
1095                1100                1105                1110

ATT GCT GGA ATC CTT TTG CTG TTA GCT CTG GTT GCA ATT TTA TGG AAG     3513
Ile Ala Gly Ile Leu Leu Leu Leu Ala Leu Val Ala Ile Leu Trp Lys
                1115                1120                1125

CTC GGC TTC TTC AAA AGA AAA TAT GAA AAG ATG ACC AAA AAT CCA GAT     3561
Leu Gly Phe Phe Lys Arg Lys Tyr Glu Lys Met Thr Lys Asn Pro Asp
            1130                1135                1140

GAG ATT GAT GAG ACC ACA GAG CTC AGT AGC TGAACCAGCA GACCTACCTG CAGT  3615
Glu Ile Asp Glu Thr Thr Glu Leu Ser Ser
        1145                1150
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GGGAACCGGC AGCATCCCAG CCAGGGTTTG CTGTTTGCGT GCATGGATTT CTTTTTAAAT 3675
CCCATATTTT TTTTATCATG TCGTAGGTAA ACTAACCTGG TATTTTAAGA GAAAACTGCA 3735
GGTCAGTTTG GATGAAGAAA TTGTGGGGGG TGGGGGAGGT GCGGGGGGCA GGTAGGGAAA 3795
TAATAGGGAA AATACCTATT TTATATGATG GGGGAAAAAA AGTAATCTTT AAACTGGCTG 3855
GCCCAGAGTT TACATTCTAA TTTGCATTGT GTCAGAAACA TGAAATGCTT CCAAGCATGA 3915
CAACTTTTAA AGAAAAATAT GATACTCTCA GATTTTAAGG GGGAAAACTG TTCTCTTTAA 3975
AATATTTGTC TTTAAACAGC AACTACAGAA GTGGAAGTGC TTGATATGTA AGTACTTCCA 4035
CTTGTGTATA TTTTAATGAA TATTGATGTT AACAAGAGGG GAAAACAAAA CACAGGTTTT 4095
TTCAATTTAT GCTGCTCATC CAAAGTTGCC ACAGATGATA CTTCCAAGTG ATAATTTTAT 4155
TTATAAACTA GGTAAAATTT GTTGTTGGTT CCTTTTATAC CACGGCTGCC CCTTCCACAC 4215
CCCATCTTGC TCTAATGATC AAAACATGCT TGAATAACTG AGCTTAGAGT ATACCTCCTA 4275
TATGTCCATT TAAGTTAGGA GAGGGGGCGA TATAGAGACT AAGGCACAAA ATTTTGTTTA 4335
AAACTCAGAA TATAACATTT ATGTAAAATC CCATCTGCTA GAAGCCCATC CTGTGCCAGA 4395
GGAAGGAAAA GGAGGAAATT TCCTTTCTCT TTTAGGAGGC ACAACAGTTC TCTTCTAGGA 4455
TTTGTTTGGC TGACTGGCAG TAACCTAGTG AATTTTTGAA AGATGAGTAA TTTCTTTGGC 4515
AACCTTCCTC CTCCCTTACT GAACCACTCT CCCACCTCCT GGTGGTACCA TTATTATAGA 4575
AGCCCTCTAC AGCCTGACTT TCTCTCCAGC GGTCCAAAGT TATCCCCTCC TTTACCCCTC 4635
ATCCAAAGTT CCCACTCCTT CAGGACAGCT GCTGTGCATT AGATATTAGG GGGGAAAGTC 4695
ATCTGTTTAA TTTACACACT TGCATGAATT ACTGTATATA AACTCCTTAA CTTCAGGGAG 4755
CTATTTTCAT TTAGTGCTAA ACAAGTAAGA AAAATAAGCT AGAGTGAATT TCTAAATGTT 4815
GGAATGTTAT GGGATGTAAA CAATGTAAAG TAAAACACTC TCAGGATTTC ACCAGAAGTT 4875
ACAGATGAGG CACTGGAAAC CACCACCAAA TTAGCAGGTG CACCTTCTGT GGCTGTCTTG 4935
TTTCTGAAGT ACTTTTTCTT CCACAAGAGT GAATTTGACC TAGGCAAGTT TGTTCAAAAG 4995
GTAGATCCTG AGATGATTTG GTCAGATTGG GATAAGGCCC AGCAATCTGC ATTTTAACAA 5055
GCACCCCAGT CACTAGGATG CAGATGGACC ACACTTTGAG AAACACCACC CATTTCTACT 5115
TTTTGCACCT TATTTTCTCT GTTCCTGAGC CCCCACATTC TCTAGGAGAA ACTTAGATTA 5175
AAATTCACAG ACACTACATA TCTAAAGCTT TGACAAGTCC TTGACCTCTA TAAACTTCAG 5235
AGTCCTCATT ATAAAATGGG AAGACTGAGC TGGAGTTCAG CAGTGATGCT TTTTAGTTTT 5295
AAAAGTCTAT GATCTGATCT GGACTTCCTA TAATACAAAT ACACAATCCT CCAAGAATTT 5355
GACTTGGAAA AGGAATTC 5373

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1181 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein
(iii) HYPOTHETICAL: NO
(iv) ANTISENSE: NO
(v) FRAGMENT TYPE: internal
(vi) ORIGINAL SOURCE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

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Met Gly Pro Glu Arg Thr Gly Ala Ala Pro Leu Pro Leu Leu Leu Val
-29             -25             -20                 -15

Leu Ala Leu Ser Gln Gly Ile Leu Asn Cys Cys Leu Ala Tyr Asn Val
            -10                 -5                  1

Gly Leu Pro Glu Ala Lys Ile Phe Ser Gly Pro Ser Ser Glu Gln Phe
      5                 10                  15
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Gly Tyr Ala Val Gln Gln Phe Ile Asn Pro Lys Gly Asn Trp Leu Leu
 20                  25                  30                  35

Val Gly Ser Pro Trp Ser Gly Phe Pro Glu Asn Arg Met Gly Asp Val
                 40                  45                  50

Tyr Lys Cys Pro Val Asp Leu Ser Thr Ala Thr Cys Glu Lys Leu Asn
             55                  60                  65

Leu Gln Thr Ser Thr Ser Ile Pro Asn Val Thr Glu Met Lys Thr Asn
         70                  75                  80

Met Ser Leu Gly Leu Ile Leu Thr Arg Asn Met Gly Thr Gly Gly Phe
     85                  90                  95

Leu Thr Cys Gly Pro Leu Trp Ala Gln Gln Cys Gly Asn Gln Tyr Tyr
100                 105                 110                 115

Thr Thr Gly Val Cys Ser Asp Ile Ser Pro Asp Phe Gln Leu Ser Ala
                120                 125                 130

Ser Phe Ser Pro Ala Thr Gln Pro Cys Pro Ser Leu Ile Asp Val Val
            135                 140                 145

Val Val Cys Asp Glu Ser Asn Ser Ile Tyr Pro Trp Asp Ala Val Lys
        150                 155                 160

Asn Phe Leu Glu Lys Phe Val Gln Gly Leu Asp Ile Gly Pro Thr Lys
    165                 170                 175

Thr Gln Val Gly Leu Ile Gln Tyr Ala Asn Asn Pro Arg Val Val Phe
180                 185                 190                 195

Asn Leu Asn Thr Tyr Lys Thr Lys Glu Glu Met Ile Val Ala Thr Ser
                200                 205                 210

Gln Thr Ser Gln Tyr Gly Gly Asp Leu Thr Asn Thr Phe Gly Ala Ile
            215                 220                 225

Gln Tyr Ala Arg Lys Tyr Ala Tyr Ser Ala Ala Ser Gly Gly Arg Arg
        230                 235                 240

Ser Ala Thr Lys Val Met Val Val Val Thr Asp Gly Glu Ser His Asp
    245                 250                 255

Gly Ser Met Leu Lys Ala Val Ile Asp Gln Cys Asn His Asp Asn Ile
260                 265                 270                 275

Leu Arg Phe Gly Ile Ala Val Leu Gly Tyr Leu Asn Arg Asn Ala Leu
                280                 285                 290

Asp Thr Lys Asn Leu Ile Lys Glu Ile Lys Ala Ile Ala Ser Ile Pro
            295                 300                 305

Thr Glu Arg Tyr Phe Phe Asn Val Ser Asp Glu Ala Ala Leu Leu Glu
        310                 315                 320
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Lys Ala Gly Thr Leu Gly Glu Gln Ile Phe Ser Ile Glu Gly Thr Val
    325                 330                 335

Gln Gly Gly Asp Asn Phe Gln Met Glu Met Ser Gln Val Gly Phe Ser
340                 345                 350                 355

Ala Asp Tyr Ser Ser Gln Asn Asp Ile Leu Met Leu Gly Ala Val Gly
                360                 365                 370

Ala Phe Gly Trp Ser Gly Thr Ile Val Gln Lys Thr Ser His Gly His
            375                 380                 385

Leu Ile Phe Pro Lys Gln Ala Phe Asp Gln Ile Leu Gln Asp Arg Asn
        390                 395                 400

His Ser Ser Tyr Leu Gly Tyr Ser Val Ala Ala Ile Ser Thr Gly Glu
    405                 410                 415

Ser Thr His Phe Val Ala Gly Ala Pro Arg Ala Asn Tyr Thr Gly Gln
420                 425                 430                 435

Ile Val Leu Tyr Ser Val Asn Glu Asn Gly Asn Ile Thr Val Ile Gln
                440                 445                 450

Ala His Arg Gly Asp Gln Ile Gly Ser Tyr Phe Gly Ser Val Leu Cys
            455                 460                 465

Ser Val Asp Val Asp Lys Asp Thr Ile Thr Asp Val Leu Leu Val Gly
        470                 475                 480

Ala Pro Met Tyr Met Ser Asp Leu Lys Lys Glu Glu Gly Arg Val Tyr
    485                 490                 495

Leu Phe Thr Ile Lys Lys Gly Ile Leu Gly Gln His Gln Phe Leu Glu
500                 505                 510                 515

Gly Pro Glu Gly Ile Glu Asn Thr Arg Phe Gly Ser Ala Ile Ala Ala
                520                 525                 530

Leu Ser Asp Ile Asn Met Asp Gly Phe Asn Asp Val Ile Val Gly Ser
            535                 540                 545

Pro Leu Glu Asn Gln Asn Ser Gly Ala Val Tyr Ile Tyr Asn Gly His
        550                 555                 560

Gln Gly Thr Ile Arg Thr Lys Tyr Ser Gln Lys Ile Leu Gly Ser Asp
    565                 570                 575

Gly Ala Phe Arg Ser His Leu Gln Tyr Phe Gly Arg Ser Leu Asp Gly
580                 585                 590                 595

Tyr Gly Asp Leu Asn Gly Asp Ser Ile Thr Asp Val Ser Ile Gly Ala
                600                 605                 610

Phe Gly Gln Val Val Gln Leu Trp Ser Gln Ser Ile Ala Asp Val Ala
            615                 620                 625
```

Ile Glu Ala Ser Phe Thr Pro Glu Lys Ile Thr Leu Val Asn Lys Asn
630 635 640

Ala Gln Ile Ile Leu Lys Leu Cys Phe Ser Ala Lys Phe Arg Pro Thr
645 650 655

Lys Gln Asn Asn Gln Val Ala Ile Val Tyr Asn Ile Thr Leu Asp Ala
660 665 670 675

Asp Gly Phe Ser Ser Arg Val Thr Ser Arg Gly Leu Phe Lys Glu Asn
680 685 690

Asn Glu Arg Cys Leu Gln Lys Asn Met Val Val Asn Gln Ala Gln Ser
695 700 705

Cys Pro Glu His Ile Ile Tyr Ile Gln Glu Pro Ser Asp Val Val Asn
710 715 720

Ser Leu Asp Leu Arg Val Asp Ile Ser Leu Glu Asn Pro Gly Thr Ser
725 730 735

Pro Ala Leu Glu Ala Tyr Ser Glu Thr Ala Lys Val Phe Ser Ile Pro
740 745 750 755

Phe His Lys Asp Cys Gly Glu Asp Gly Leu Cys Ile Ser Asp Leu Val
760 765 770

Leu Asp Val Arg Gln Ile Pro Ala Ala Gln Glu Gln Pro Phe Ile Val
775 780 785

Ser Asn Gln Asn Lys Arg Leu Thr Phe Ser Val Thr Leu Lys Asn Lys
790 795 800

Arg Glu Ser Ala Tyr Asn Thr Gly Ile Val Val Asp Phe Ser Glu Asn
805 810 815

Leu Phe Phe Ala Ser Phe Ser Leu Pro Val Asp Gly Thr Glu Val Thr
820 825 830 835

Cys Gln Val Ala Ala Ser Gln Lys Ser Val Ala Cys Asp Val Gly Tyr
840 845 850

Pro Ala Leu Lys Arg Glu Gln Gln Val Thr Phe Thr Ile Asn Phe Asp
855 860 865

Phe Asn Leu Gln Asn Leu Gln Asn Gln Ala Ser Leu Ser Phe Gln Ala
870 875 880

Leu Ser Glu Ser Gln Glu Glu Asn Lys Ala Asp Asn Leu Val Asn Leu
885 890 895

Lys Ile Pro Leu Leu Tyr Asp Ala Glu Ile His Leu Thr Arg Ser Thr
900 905 910 915

Asn Ile Asn Phe Tyr Glu Ile Ser Ser Asp Gly Asn Val Pro Ser Ile
920 925 930

```
Val His Ser Phe Glu Asp Val Gly Pro Lys Phe Ile Phe Ser Leu Lys
            935                 940                 945

Val Thr Thr Gly Ser Val Pro Val Ser Met Ala Thr Val Ile Ile His
        950                 955                 960

Ile Pro Gln Tyr Thr Lys Glu Lys Asn Pro Leu Met Tyr Leu Thr Gly
    965                 970                 975

Val Gln Thr Asp Lys Ala Gly Asp Ile Ser Cys Asn Ala Asp Ile Asn
980                 985                 990                 995

Pro Leu Lys Ile Gly Gln Thr Ser Ser Ser Val Ser Phe Lys Ser Glu
               1000                1005                1010

Asn Phe Arg His Thr Lys Glu Leu Asn Cys Arg Thr Ala Ser Cys Ser
            1015                1020                1025

Asn Val Thr Cys Trp Leu Lys Asp Val His Met Lys Gly Glu Tyr Phe
        1030                1035                1040

Val Asn Val Thr Thr Arg Ile Trp Asn Gly Thr Phe Ala Ser Ser Thr
    1045                1050                1055

Phe Gln Thr Val Gln Leu Thr Ala Ala Ala Glu Ile Asn Thr Tyr Asn
1060                1065                1070                1075

Pro Glu Ile Tyr Val Ile Glu Asp Asn Thr Val Thr Ile Pro Leu Met
               1080                1085                1090

Ile Met Lys Pro Asp Glu Lys Ala Glu Val Pro Thr Gly Val Ile Ile
            1095                1100                1105

Gly Ser Ile Ile Ala Gly Ile Leu Leu Leu Leu Ala Leu Val Ala Ile
        1110                1115                1120

Leu Trp Lys Leu Gly Phe Phe Lys Arg Lys Tyr Glu Lys Met Thr Lys
    1125                1130                1135

Asn Pro Asp Glu Ile Asp Glu Thr Thr Glu Leu Ser Ser
1140                1145                1150
```

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA
(iii) HYPOTHETICAL: NO
(iv) ANTISENSE: NO
(v) FRAGMENT TYPE:
(vi) ORIGINAL SOURCE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

CCAGAGTCAC TCTCACAGAG 20

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA
(iii) HYPOTHETICAL: NO
(iv) ANTISENSE: NO
(v) FRAGMENT TYPE:
(vi) ORIGINAL SOURCE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

CACAGCGTAC ACGTACACC 19

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA
(iii) HYPOTHETICAL: NO
(iv) ANTISENSE: NO
(v) FRAGMENT TYPE:
(vi) ORIGINAL SOURCE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

CACTTATAGA CATCTCCAG 19

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA
(iii) HYPOTHETICAL: NO
(iv) ANTISENSE: NO
(v) FRAGMENT TYPE:
(vi) ORIGINAL SOURCE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

CATCCATGTT GATGTCTG 18

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA
(iii) HYPOTHETICAL: NO
(iv) ANTISENSE: NO
(v) FRAGMENT TYPE:
(vi) ORIGINAL SOURCE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

CATGTGATTC ACCGTCAG 18

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA
(iii) HYPOTHETICAL: NO
(iv) ANTISENSE: NO
(v) FRAGMENT TYPE:
(vi) ORIGINAL SOURCE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

GCATATTGAA TTGCTCCGAA TGTG 24

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA
(iii) HYPOTHETICAL: NO
(iv) ANTISENSE: NO
(v) FRAGMENT TYPE:
(vi) ORIGINAL SOURCE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

GGCGTATGCA CAACGCA 17

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA
(iii) HYPOTHETICAL: NO
(iv) ANTISENSE: NO
(v) FRAGMENT TYPE:
(vi) ORIGINAL SOURCE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

GCGACAGCTG ACCAGTCAGC A 21

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA
(iii) HYPOTHETICAL: NO
(iv) ANTISENSE: NO
(v) FRAGMENT TYPE:
(vi) ORIGINAL SOURCE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

CACTCCTCCA CAGCTCCT 18

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA
(iii) HYPOTHETICAL: NO
(iv) ANTISENSE: NO
(v) FRAGMENT TYPE:
(vi) ORIGINAL SOURCE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

ACATGTACTC ACTGG 15

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA
(iii) HYPOTHETICAL: NO
(iv) ANTISENSE: NO
(v) FRAGMENT TYPE:
(vi) ORIGINAL SOURCE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

CTCACATGTG GTCCTCTG 18

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA
(iii) HYPOTHETICAL: NO
(iv) ANTISENSE: NO
(v) FRAGMENT TYPE:
(vi) ORIGINAL SOURCE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

GTCCTGTTGA CCTATCCACT GC 22

WE CLAIM:

1. A method to identify a mammal having or at risk for developing glomerulopathy comprising the steps of:

analyzing a tissue sample from a mammal known to contain cells expressing integrin RNA or protein for integrin subunit expression; and

comparing integrin subunit expression in the sample with a control tissue sample, wherein altered integrin subunit expression is correlated with glomerulopathy.

2. The method of Claim 1, wherein the mammal is a human.

3. The method of Claim 1, wherein the tissue sample is a kidney biopsy.

4. The method of Claim 1, wherein the tissue sample is blood.

5. The method of Claim 4, wherein the blood sample contains polymorphonuclear cells or monocytes.

6. The method of Claim 1, wherein the tissue sample is a skin biopsy.

7. The method of Claim 1, wherein said analysis comprises *in situ* hybridization.

8. The method of Claim 7, wherein said *in situ* hybridization comprises PCR enhanced *in situ* hybridization.

9. The method of Claim 1, wherein said analyzing comprises isolating RNA from the sample.

10. The method of Claim 1, wherein said analyzing comprises performing PCR, detecting amplified fragments from an integrin subunit and comparing the amount of amplified fragments to the amount of amplified fragments obtained from the control.

11. The method of claim 1, wherein the integrin subunit is an alpha integrin subunit.

12. The method of Claim 11, wherein the α integrin subunit is α1, α2, α3, or α5 integrin subunit.

13. The method of claim 12, wherein the α integrin subunit is α1 or α2 integrin subunit.

14. The method of claim 1, wherein a decrease in α1 integrin subunit in the tissue sample as compared with control tissue is correlated with nephropathy.

15. The method of claim 1, wherein an increase in α2 α3, α5, or β1 integrin subunit in the tissue sample as compared with control tissue is correlated with nephropathy.

16. The method of claim 1, wherein an increase in α2 and a decrease in α1 integrin subunit in the tissue sample as compared with control tissue is correlated with nephropathy.

17. The method of Claim 7, wherein a nucleic acid probe is used to detect integrin, and the probe comprises a 3.9kb fragment of α1 from the 5' end to nucleotide 3900.

18. The method of Claim 7, wherein a nucleic acid probe is used to detect integrin, and the probe comprises a 1.8kb fragment of α2 from 5' end through the EcoRI site at nucleotide 1800.

19. The method of Claim 1, wherein said analyzing comprises incubating the sample with an anti-integrin subunit antibody.

20. The method of Claim 1, wherein the nondiabetic control sample is from a mammal with no history of hypertension.

21. The method of Claim 1, wherein an increase of about 25% - 100% in the level of α2 integrin subunit expression in the sample tissue as compared with the control is correlated with nephropathy.

22. The method of Claim 1, wherein a decrease of about 25% - 100% in the level of α1 integrin subunit expression in the sample tissue as compared with the control is correlated with nephropathy.

23. A method to identify a mammal having or at risk for developing glomerulopathy comprising the steps of:

analyzing a tissue sample from a mammal known to contain cells expressing integrin protein for α1 and α2 integrin subunit expression as compared with a control tissue sample; and

correlating a decreased level of α1 integrin subunit expression and/or an increased level of α2 integrin subunit expression in the sample tissue as compared to the control with nephropathy.

24. A method to identify a mammal with diabetes who has or is at risk for developing secondary pathological changes associated with diabetes comprising the steps of:

analyzing a tissue sample from a mammal known to contain cells expressing integrin protein for integrin subunit expression; and

correlating alterations in the level of expression of least one integrin subunit as compared with a control tissue sample with the presence of or the risk for developing secondary pathological changes associated with diabetes.

25. The method of claim 25, wherein said integrin subunit is α1, α2, α3, α5, or β1.

26. The method of claim 25, wherein said integrin subunit is α1 or α2.

27. The method of claim 1, wherein said comparing of a sample with a control comprises comparing a first sample obtained from an individual with a second sample obtained from the same individual at a later sampling time.

28. A kit for the diagnosis of nephropathy comprising:

two sets of hybridization probes or antibodies capable of detecting each of α1 and α2 integrin subunit expression in a tissue sample.

29. The kit of claim 28 further comprising primer sets for the amplification of α1 and α2 integrin subunits.

30. The kit of claim 28 further comprising control, standard α1 and α2 integrin subunits.

PCT

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International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
C12Q 1/68, C07K 14/705, 16/28, G01N 33/566

A1

(11) International Publication Number: WO 97/04133

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60/001,861	3 August 1995 (03.08.95)	US
60/016,700	2 May 1996 (02.05.96)	US

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(81) Designated States: AL, AM, AT, AT (Utility model), AU, AZ, BB, BG, BR, BY, CA, CH, CN, CU, CZ, CZ (Utility model), DE, DE (Utility model), DK, DK (Utility model), EE, EE (Utility model), ES, FI, FI (Utility model), GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SK (Utility model), TJ, TM, TR, TT, UA, UG, US, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published

With international search report.

Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: ANALYSIS OF ALPHA INTEGRINS FOR THE DIAGNOSIS OF DIABETIC NEPHROPATHY

(57) Abstract

Analysis of alterations in integrin subunit expression, particularly $\alpha 1$ and/or $\alpha 2$ integrin subunit expression from integrin producing cells as compared to normal controls as a diagnostic method to identify individuals who have or are predisposed to pathologies associated with altered matrix deposition, such as diabetic renal nephropathy.

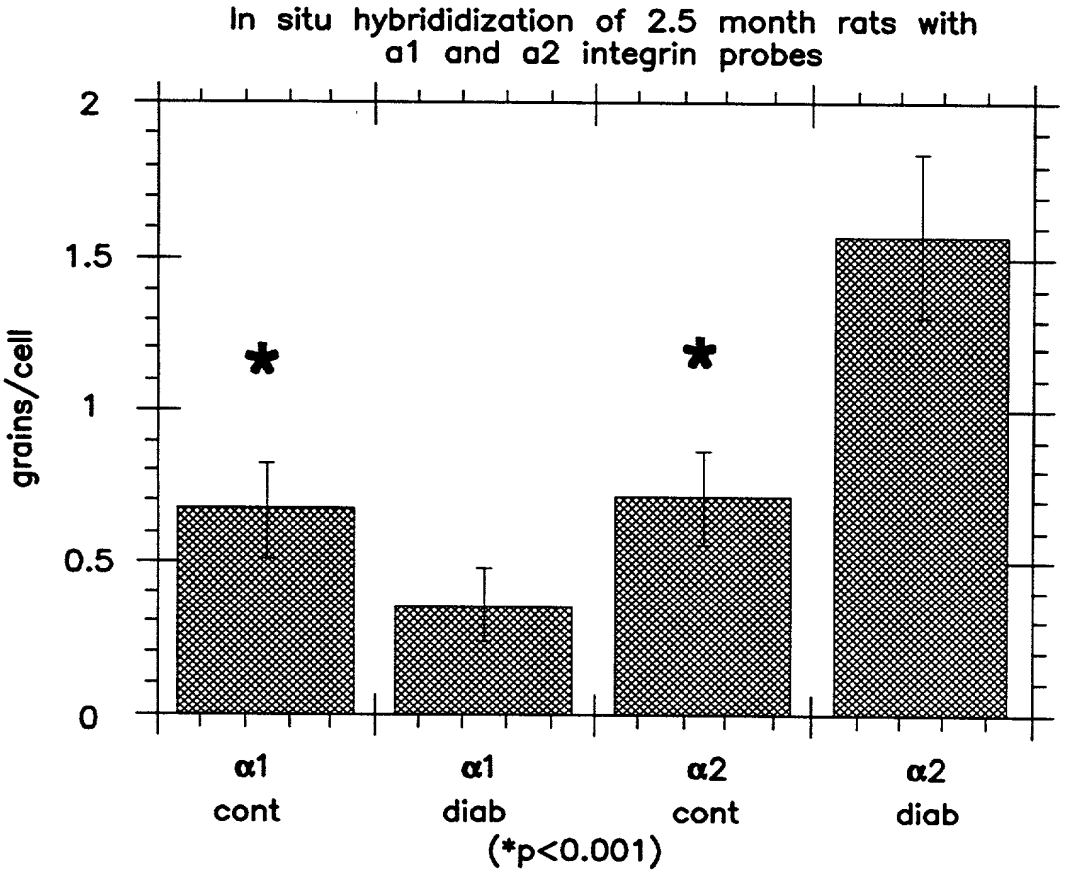
In situ hybridididization of 2.5 month rats with
a1 and a2 integrin probes
grains/cell
2
1.5
1
0.5
0
*
*
α1
cont
α1
diab
α2
cont
α2
diab
(*p<0.001)

FIG. 1

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	TSILIBARY, ET AL.	Examiner:	UNKNOWN
Serial No.:	09/000,004	Group Art Unit:	UNKNOWN
Filed:	JANUARY 21, 1998	Docket No.:	600.314USWO
Title:	ANALYSIS OF ALPHA INTEGRINS FOR THE DIAGNOSIS OF DIABETIC NEPHROPATHY		

CERTIFICATE UNDER 37 CFR 1.8:
I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail, with sufficient postage, in an envelope addressed to. BOX PCT, Commissioner for Patents, Washington, D C 20231 on May 23, 2001.

Anne Harrison
ANNE HARRISON

CHANGE OF ADDRESS

BOX PCT
Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

Please change the correspondence address for the above-referenced patent application to:

Merchant & Gould P.C.
P.O. Box 2903
Minneapolis, Minnesota 55402-0903

If there are any questions regarding this matter, please call the undersigned at 612.332.5300.

Respectfully submitted,

MERCHANT & GOULD P.C.
P.O. Box 2910
Minneapolis, Minnesota 55402-0910
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Date: May 23, 2001

Denise M. Kettelberger
Denise M. Kettelberger, Ph.D.
Reg. No. 33,924
DMK:KC:PSTalh

#10

Attorney Docket No. 600.314USWO

MERCHANT, GOULD, SMITH, EDELL, WELTER & SCHMIDT

United States Patent Application

COMBINED DECLARATION AND POWER OF ATTORNEY

As a below named inventor I hereby declare that: my residence, post office address and citizenship are as stated below next to my name; that

I verily believe I am the original, first and sole inventor (if only one name is listed below) or a joint inventor (if plural inventors are named below) of the subject matter which is claimed and for which a patent is sought on the invention entitled: ANALYSIS OF ALPHA INTEGRINS FOR THE DIAGNOSIS OF DIABETIC NEPHROPATHY

The specification of which
a. ☐ is attached hereto
b. ☒ was filed on January 21, 1998 as application serial no. and was amended on (if applicable) (in the case of a PCT-filed application) described and claimed in international no. PCT/US96/12067 filed 19 July 1996 and as amended on (if any), which I have reviewed and for which I solicit a United States patent.

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the patentability of this application in accordance with Title 37, Code of Federal Regulations, § 1.56 (attached hereto).

I hereby claim foreign priority benefits under Title 35, United States Code, § 119/365 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on the basis of which priority is claimed:

a. ☐ no such applications have been filed.
b. ☐ such applications have been filed as follows:

FOREIGN APPLICATION(S), IF ANY, CLAIMING PRIORITY UNDER 35 USC § 119			
COUNTRY	**APPLICATION NUMBER**	**DATE OF FILING (day, month, year)**	**DATE OF ISSUE (day, month, year)**
ALL FOREIGN APPLICATION(S), IF ANY, FILED BEFORE THE PRIORITY APPLICATION(S)			
COUNTRY	**APPLICATION NUMBER**	**DATE OF FILING (day, month, year)**	**DATE OF ISSUE (day, month, year)**

I hereby claim the benefit under Title 35, United States Code, § 120/365 of any United States and PCT international application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, § 1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application.

U.S. APPLICATION NUMBER	DATE OF FILING (day, month, year)	STATUS (patented, pending, abandoned)

I hereby claim the benefit under Title 35, United States Code § 119(e) of any United States provisional application(s) listed below:

U.S. PROVISIONAL APPLICATION NUMBER	DATE OF FILING (Day, Month, Year)
60/001,387	July 21, 1995
60/001,861	August 3, 1995
60/016,700	May 2, 1996

I hereby appoint the following attorney(s) and/or patent agent(s) to prosecute this application and to transact all business in the Patent and Trademark Office connected herewith:

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DiPietro, Mark J.	Reg. No. 28,707	Sebald, Gregory A.	Reg. No. 33,280
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Farber, Michael B.	Reg. No. 32,612	Smith, Jerome R.	Reg. No. 35,684
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Kowalchyk, Katherine M.	Reg. No. 36,848	Xu, Min S.	Reg. No. 39,536
Lacy, Paul E.	Reg. No. 38,946		
Larson, James A.	Reg. No. 40,443		

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Welter & Schmidt
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Minneapolis, MN 55402-4131

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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Signature of Inventor 202:			**Date:** 4/10/98	

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Signature of Inventor 203: Suman Setty			**Date:** 4·6·98	

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Signature of Inventor 204: Michael Mauer			**Date:** 4.6.98	

§ 1.56 Duty to disclose information material to patentability.

(a) A patent by its very nature is affected with a public interest. The public interest is best served, and the most effective patent examination occurs when, at the time an application is being examined, the Office is aware of and evaluates the teachings of all information material to patentability. Each individual associated with the filing and prosecution of a patent application has a duty of candor and good faith in dealing with the Office, which includes a duty to disclose to the Office all information known to that individual to be material to patentability as defined in this section. The duty to disclose information exists with respect to each pending claim until the claim is canceled or withdrawn from consideration, or the application becomes abandoned. Information material to the patentability of a claim that is canceled or withdrawn from consideration need not be submitted if the information is not material to the patentability of any claim remaining under consideration in the application. There is no duty to submit information which is not material to the patentability of any existing claim. The duty to disclose all information known to be material to patentability is deemed to be satisfied if all information known to be material to patentability of any claim issued in a patent was cited by the Office or submitted to the Office in the manner prescribed by §§ 1.97(b)-(d) and 1.98. However, no patent will be granted on an application in connection with which fraud on the Office was practiced or attempted or the duty of disclosure was violated through bad faith or intentional misconduct. The Office encourages applicants to carefully examine:

(1) prior art cited in search reports of a foreign patent office in a counterpart application, and

(2) the closest information over which individuals associated with the filing or prosecution of a patent application believe any pending claim patentably defines, to make sure that any material information contained therein is disclosed to the Office.

(b) Under this section, information is material to patentability when it is not cumulative to information already of record or being made of record in the application, and

(1) It establishes, by itself or in combination with other information, a prima facie case of unpatentability of a claim; or

(2) It refutes, or is inconsistent with, a position the applicant takes in:

(i) Opposing an argument of unpatentability relied on by the Office, or

(ii) Asserting an argument of patentability.

A prima facie case of unpatentability is established when the information compels a conclusion that a claim is unpatentable under the preponderance of evidence, burden-of-proof standard, giving each term in the claim its broadest reasonable construction consistent with the specification, and before any consideration is given to evidence which may be submitted in an attempt to establish a contrary conclusion of patentability.

(c) Individuals associated with the filing or prosecution of a patent application within the meaning of this section are:

(1) Each inventor named in the application:

(2) Each attorney or agent who prepares or prosecutes the application; and

(3) Every other person who is substantively involved in the preparation or prosecution of the application and who is associated with the inventor, with the assignee or with anyone to whom there is an obligation to assign the application.

(d) Individuals other than the attorney, agent or inventor may comply with this section by disclosing information to the attorney, agent, or inventor.

SEQUENCE LISTING

<110> Tsilibary, Photini-Effie
Charonis, Aristidis S.
Setty, Suman
Mauer, Michael

<120> ANALYSIS OF ALPHA INTEGRINS FOR THE DIAGNOSIS OF DIABETIC NEPHROPATHY

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Val Lys Asn Ser Met Ser Phe Ser Gly Pro Val Glu Asp Met Phe Gly
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Tyr Thr Val Gln Gln Tyr Glu Asn Glu Glu Gly Lys Trp Val Leu Ile
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Gly Ser Pro Leu Val Gly Gln Pro Lys Ala Arg Thr Gly Asp Val Tyr
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Lys Cys Pro Val Gly Arg Glu Arg Ala Met Pro Cys Val Lys Leu Asp
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Leu Pro Val Asn Thr Ser Ile Pro Asn Val Thr Glu Ile Lys Glu Asn
            100                 105                 110

atg aca ttt gga tca act tta gtc acc aac ccg aat gga gga ttt ctg      803
Met Thr Phe Gly Ser Thr Leu Val Thr Asn Pro Asn Gly Gly Phe Leu
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gca tgt ggg ccc ttg tat gcc tat aga tgt gga cat ttg cat tat aca      851
Ala Cys Gly Pro Leu Tyr Ala Tyr Arg Cys Gly His Leu His Tyr Thr
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act gga ata tgt tct gat gtc agt cct aca ttt caa gtt gtg aac tcc      899
Thr Gly Ile Cys Ser Asp Val Ser Pro Thr Phe Gln Val Val Asn Ser
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Phe Ala Pro Val Gln Glu Cys Ser Thr Gln Leu Asp Ile Val Ile Val
                165                 170                 175

ctg gat ggc tcc aac agc atc tac ccc tgg gaa agt gtc atc gcc ttt      995
Leu Asp Gly Ser Asn Ser Ile Tyr Pro Trp Glu Ser Val Ile Ala Phe
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Asn Lys Tyr Ser Ser Thr Glu Glu Val Leu Val Ala Ala Asn Lys Ile
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ggc cga cag gga ggc ctc caa acg atg aca gcc ctt gga ata gac aca     1187
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Lys Lys Val Met Val Ile Val Thr Asp Gly Glu Ser His Asp Asn Tyr
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cgc ctg aaa cag gtc atc caa gac tgc gag gac gaa aac att cag cga      1331
Arg Leu Lys Gln Val Ile Gln Asp Cys Glu Asp Glu Asn Ile Gln Arg
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Lys Ala Leu Gly Glu Arg Ile Phe Ala Leu Glu Ala Thr Ala Asp Gln
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Ser Ala Ala Ser Phe Glu Met Glu Met Ser Gln Thr Gly Phe Ser Ala
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cac tac tcc cag gac tgg gtc atg ctt gga gcg gtg gga gcc tat gac      1619
His Tyr Ser Gln Asp Trp Val Met Leu Gly Ala Val Gly Ala Tyr Asp
385                 390                 395                 400

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Trp Asn Gly Thr Val Val Met Gln Lys Ala Asn Gln Met Val Ile Pro
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cat aac acc acc ttt caa act gag ccc gcc aag atg aac gag cct ctg      1715
His Asn Thr Thr Phe Gln Thr Glu Pro Ala Lys Met Asn Glu Pro Leu
            420                 425                 430

gct tct tat tta ggt tac aca gtg aac tcg gcc acc atc cct gga gat      1763
Ala Ser Tyr Leu Gly Tyr Thr Val Asn Ser Ala Thr Ile Pro Gly Asp
        435                 440                 445

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Val Leu Tyr Ile Ala Gly Gln Pro Arg Tyr Asn His Thr Gly Gln Val
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Val Ile Tyr Lys Met Glu Asp Gly Asn Ile Asn Ile Leu Gln Thr Leu
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Met Tyr Met Gly Thr Glu Lys Glu Glu Gln Gly Lys Val Tyr Val Tyr
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Ala Val Asn Gln Thr Arg Phe Glu Tyr Gln Met Ser Leu Glu Pro Ile
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agg cag acc tgc tgc tca tcc ctg aag gat aat tca tgc acg aaa gaa     2099
Arg Gln Thr Cys Cys Ser Ser Leu Lys Asp Asn Ser Cys Thr Lys Glu
545                 550                 555                 560

aac aag aat gag ccc tgc ggg gcc cgc ttc gga aca gca att gct gct     2147
Asn Lys Asn Glu Pro Cys Gly Ala Arg Phe Gly Thr Ala Ile Ala Ala
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gta aaa gac ctc aac gtg gat gga ttt aat gac gtc gtg att gga gct     2195
Val Lys Asp Leu Asn Val Asp Gly Phe Asn Asp Val Val Ile Gly Ala
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ccg ctg gaa gat gac cac gca gga gct gtg tac att tat cat ggc agt     2243
Pro Leu Glu Asp Asp His Ala Gly Ala Val Tyr Ile Tyr His Gly Ser
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Gly Lys Thr Ile Arg Glu Ala Tyr Ala Gln Arg Ile Pro Ser Gly Gly
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gat tta aat ggt gac ggt ctg act gac gtg acc att gga ggc ctt ggt     2387
Asp Leu Asn Gly Asp Gly Leu Thr Asp Val Thr Ile Gly Gly Leu Gly
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gga gca gcc ctc ttc tgg gcc aga gat gtg gct gta gtt aaa gtg acc     2435
Gly Ala Ala Leu Phe Trp Ala Arg Asp Val Ala Val Val Lys Val Thr
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atg aat ttt gaa ccc aat aaa gtg aat att caa aag aaa aac tgc cgt     2483
Met Asn Phe Glu Pro Asn Lys Val Asn Ile Gln Lys Lys Asn Cys Arg
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gtg gag ggc aaa gaa aca gtg tgc ata aat gct aca atg tgt ttt cat     2531
Val Glu Gly Lys Glu Thr Val Cys Ile Asn Ala Thr Met Cys Phe His
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gtg aaa tta aag tct aaa gag gac tca att tac gag gct gat ctg cag     2579
Val Lys Leu Lys Ser Lys Glu Asp Ser Ile Tyr Glu Ala Asp Leu Gln
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Ser Glu Cys Ile Arg His Ser Phe Tyr Met Leu Asp Lys His Asp Phe
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cag gac tct gtg aga gtg act ctg gat ttt aat ctc act gat cca gaa      2771
Gln Asp Ser Val Arg Val Thr Leu Asp Phe Asn Leu Thr Asp Pro Glu
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aat ggt cct gta ctt gat gac gct ctg cca aac tca gtc cac gaa cac      2819
Asn Gly Pro Val Leu Asp Asp Ala Leu Pro Asn Ser Val His Glu His
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att ccc ttt gcc aaa gac tgt gga aac aag gaa aga tgc att tca gac      2867
Ile Pro Phe Ala Lys Asp Cys Gly Asn Lys Glu Arg Cys Ile Ser Asp
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ctc act ctg aat gtg tcc acc aca gaa aag agc ctg ctg atc gtc aag      2915
Leu Thr Leu Asn Val Ser Thr Thr Glu Lys Ser Leu Leu Ile Val Lys
            820                 825                 830

tcc cag cat gac aag ttc aac gtt agc ctc acc gtc aaa aac aaa gga      2963
Ser Gln His Asp Lys Phe Asn Val Ser Leu Thr Val Lys Asn Lys Gly
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gac agt gcg tac aac acc agg aca gtg gtg cag cat tca cca aat ctg      3011
Asp Ser Ala Tyr Asn Thr Arg Thr Val Val Gln His Ser Pro Asn Leu
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Ile Phe Ser Gly Ile Glu Glu Ile Gln Lys Asp Ser Cys Glu Ser Asn
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caa aat atc act tgc aga gtt gga tat cct ttc cta aga gca gga gaa      3107
Gln Asn Ile Thr Cys Arg Val Gly Tyr Pro Phe Leu Arg Ala Gly Glu
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acg gtt acc ttc aaa ata ata ttc cag ttt aac aca tcc cat ctc tcg      3155
Thr Val Thr Phe Lys Ile Ile Phe Gln Phe Asn Thr Ser His Leu Ser
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gaa aat gca atc att cac tta agt gca aca agt gac agt gag gag ccc      3203
Glu Asn Ala Ile Ile His Leu Ser Ala Thr Ser Asp Ser Glu Glu Pro
        915                 920                 925

ctg gaa tct ctt aat gat aat gaa gta aat att tcc atc cca gta aaa      3251
Leu Glu Ser Leu Asn Asp Asn Glu Val Asn Ile Ser Ile Pro Val Lys
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tat gaa gtt gga ctg cag ttt tac agt tct gcg agt gaa cat cac att      3299
Tyr Glu Val Gly Leu Gln Phe Tyr Ser Ser Ala Ser Glu His His Ile
945                 950                 955                 960

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His Phe Pro Met Pro Glu Leu Gln  Leu Ser Ile Ser Phe  Pro Asn Leu
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acg gca  gat ggt tat cct gta  ctg tac cca att gga  tgg tca tct      3488
Thr Ala  Asp Gly Tyr Pro Val  Leu Tyr Pro Ile Gly  Trp Ser Ser
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tca gat  aat gtg aac tgt aga  ccc cgg agc ctt gag  gac ccc ttt      3533
Ser Asp  Asn Val Asn Cys Arg  Pro Arg Ser Leu Glu  Asp Pro Phe
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Ala Thr  Ile Thr Cys Ser Leu  Leu Pro Ser Asp Leu  Ser Gln Val
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Trp Val  Ile Leu Leu Ser Ala  Phe Ala Gly Leu Leu  Leu Leu Met
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Leu Leu  Ile Leu Ala Leu Trp  Lys Ile Gly Phe Phe  Lys Arg Pro
    1160                1165                1170

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Val Lys Asn Ser Met Ser Phe Ser Gly Pro Val Glu Asp Met Phe Gly
        35                  40                  45

Tyr Thr Val Gln Gln Tyr Glu Asn Glu Glu Gly Lys Trp Val Leu Ile
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Gly Ser Pro Leu Val Gly Gln Pro Lys Ala Arg Thr Gly Asp Val Tyr
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Lys Cys Pro Val Gly Arg Glu Arg Ala Met Pro Cys Val Lys Leu Asp
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Leu Pro Val Asn Thr Ser Ile Pro Asn Val Thr Glu Ile Lys Glu Asn
            100                 105                 110

Met Thr Phe Gly Ser Thr Leu Val Thr Asn Pro Asn Gly Gly Phe Leu
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Ala Cys Gly Pro Leu Tyr Ala Tyr Arg Cys Gly His Leu His Tyr Thr
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Thr Gly Ile Cys Ser Asp Val Ser Pro Thr Phe Gln Val Val Asn Ser
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Glu Lys Phe Val Glu Glu Ile Lys Ser Ile Ala Ser Glu Pro Thr Glu
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Glu Arg Thr Gly Ala Ala Pro Leu Pro Leu Leu Leu Val Leu Ala Leu
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Ser Gln Gly Ile Leu Asn Cys Cys Leu Ala Tyr Asn Val Gly Leu Pro
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Glu Ala Lys Ile Phe Ser Gly Pro Ser Ser Glu Gln Phe Gly Tyr Ala
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Val Gln Gln Phe Ile Asn Pro Lys Gly Asn Trp Leu Leu Val Gly Ser
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ccc tgg agt ggc ttt cct gag aac cga atg gga gat gtg tat aaa tgt      297
Pro Trp Ser Gly Phe Pro Glu Asn Arg Met Gly Asp Val Tyr Lys Cys
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cct gtt gac cta tcc act gcc aca tgt gaa aaa cta aat ttg caa act      345
Pro Val Asp Leu Ser Thr Ala Thr Cys Glu Lys Leu Asn Leu Gln Thr
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tca aca agc att cca aat gtt act gag atg aaa acc aac atg agc ctc      393
Ser Thr Ser Ile Pro Asn Val Thr Glu Met Lys Thr Asn Met Ser Leu
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ggc ttg atc ctc acc agg aac atg gga act gga ggt ttt ctc aca tgt      441
Gly Leu Ile Leu Thr Arg Asn Met Gly Thr Gly Gly Phe Leu Thr Cys
                120                 125                 130

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Gly Pro Leu Trp Ala Gln Gln Cys Gly Asn Gln Tyr Tyr Thr Thr Gly
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Val Cys Ser Asp Ile Ser Pro Asp Phe Gln Leu Ser Ala Ser Phe Ser
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gat gaa tca aat agt att tat cct tgg gat gca gta aag aat ttt ttg      633
Asp Glu Ser Asn Ser Ile Tyr Pro Trp Asp Ala Val Lys Asn Phe Leu
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Glu Lys Phe Val Gln Gly Leu Asp Ile Gly Pro Thr Lys Thr Gln Val
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Gly Leu Ile Gln Tyr Ala Asn Asn Pro Arg Val Val Phe Asn Leu Asn
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Thr Tyr Lys Thr Lys Glu Glu Met Ile Val Ala Thr Ser Gln Thr Ser
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caa tat ggt ggg gac ctc aca aac aca ttc gga gca att caa tat gca      825
Gln Tyr Gly Gly Asp Leu Thr Asn Thr Phe Gly Ala Ile Gln Tyr Ala
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aga aaa tat gcc tat tca gca gct tct ggt ggg cga cga agt gct acg      873
Arg Lys Tyr Ala Tyr Ser Ala Ala Ser Gly Gly Arg Arg Ser Ala Thr
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aaa gta atg gta gtt gta act gac ggt gaa tca cat gat ggt tca atg      921
Lys Val Met Val Val Val Thr Asp Gly Glu Ser His Asp Gly Ser Met
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ttg aaa gct gtg att gat caa tgc aac cat gac aat ata ctg agg ttt      969
Leu Lys Ala Val Ile Asp Gln Cys Asn His Asp Asn Ile Leu Arg Phe
            295                 300                 305

ggc ata gca gtt ctt ggg tac tta aac aga aac gcc ctt gat act aaa     1017
Gly Ile Ala Val Leu Gly Tyr Leu Asn Arg Asn Ala Leu Asp Thr Lys
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aat tta ata aaa gaa ata aaa gcg atc gct agt att cca aca gaa aga     1065
Asn Leu Ile Lys Glu Ile Lys Ala Ile Ala Ser Ile Pro Thr Glu Arg
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tac ttt ttc aat gtg tct gat gaa gca gct cta cta gaa aag gct ggg     1113
Tyr Phe Phe Asn Val Ser Asp Glu Ala Ala Leu Leu Glu Lys Ala Gly
340                 345                 350                 355

aca tta gga gaa caa att ttc agc att gaa ggt act gtt caa gga gga     1161
Thr Leu Gly Glu Gln Ile Phe Ser Ile Glu Gly Thr Val Gln Gly Gly
                360                 365                 370

gac aac ttt cag atg gaa atg tca caa gtg gga ttc agt gca gat tac     1209
Asp Asn Phe Gln Met Glu Met Ser Gln Val Gly Phe Ser Ala Asp Tyr
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tct tct caa aat gat att ctg atg ctg ggt gca gtg gga gct ttt ggc     1257
Ser Ser Gln Asn Asp Ile Leu Met Leu Gly Ala Val Gly Ala Phe Gly
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Trp Ser Gly Thr Ile Val Gln Lys Thr Ser His Gly His Leu Ile Phe
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cct aaa caa gcc ttt gac caa att ctg cag gac aga aat cac agt tca     1353
Pro Lys Gln Ala Phe Asp Gln Ile Leu Gln Asp Arg Asn His Ser Ser
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tat tta ggt tac tct gtg gct gca att tct act gga gaa agc act cac     1401
Tyr Leu Gly Tyr Ser Val Ala Ala Ile Ser Thr Gly Glu Ser Thr His
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Phe Val Ala Gly Ala Pro Arg Ala Asn Tyr Thr Gly Gln Ile Val Leu
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tat agt gtg aat gag aat ggc aat atc acg gtt att cag gct cac cga     1497
Tyr Ser Val Asn Glu Asn Gly Asn Ile Thr Val Ile Gln Ala His Arg
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ggt gac cag att ggc tcc tat ttt ggt agt gtg ctg tgt tca gtt gat     1545
Gly Asp Gln Ile Gly Ser Tyr Phe Gly Ser Val Leu Cys Ser Val Asp
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gtg gat aaa gac acc att aca gac gtg ctc ttg gta ggt gca cca atg     1593
Val Asp Lys Asp Thr Ile Thr Asp Val Leu Leu Val Gly Ala Pro Met
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tac atg agt gac cta aag aaa gag gaa gga aga gtc tac ctg ttt act     1641
Tyr Met Ser Asp Leu Lys Lys Glu Glu Gly Arg Val Tyr Leu Phe Thr
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atc aaa aag ggc att ttg ggt cag cac caa ttt ctt gaa ggc ccc gag     1689
Ile Lys Lys Gly Ile Leu Gly Gln His Gln Phe Leu Glu Gly Pro Glu
            535                 540                 545

ggc att gaa aac act cga ttt ggt tca gca att gca gct ctt tca gac     1737
Gly Ile Glu Asn Thr Arg Phe Gly Ser Ala Ile Ala Ala Leu Ser Asp
        550                 555                 560

atc aac atg gat ggc ttt aat gat gtg att gtt ggt tca cca cta gaa     1785
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aat cag aat tct gga gct gta tac att tac aat ggt cat cag ggc act     1833
Asn Gln Asn Ser Gly Ala Val Tyr Ile Tyr Asn Gly His Gln Gly Thr
580                 585                 590                 595

atc cgc aca aag tat tcc cag aaa atc ttg gga tcc gat gga gcc ttt     1881
Ile Arg Thr Lys Tyr Ser Gln Lys Ile Leu Gly Ser Asp Gly Ala Phe
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agg agc cat ctc cag tac ttt ggg agg tcc ttg gat ggc tat gga gat     1929
Arg Ser His Leu Gln Tyr Phe Gly Arg Ser Leu Asp Gly Tyr Gly Asp
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tta aat ggg gat tcc atc acc gat gtg tct att ggt gcc ttt gga caa     1977
Leu Asn Gly Asp Ser Ile Thr Asp Val Ser Ile Gly Ala Phe Gly Gln
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Val Val Gln Leu Trp Ser Gln Ser Ile Ala Asp Val Ala Ile Glu Ala
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tca ttc aca cca gaa aaa atc act ttg gtc aac aag aat gct cag ata      2073
Ser Phe Thr Pro Glu Lys Ile Thr Leu Val Asn Lys Asn Ala Gln Ile
660                 665                 670                 675

att ctc aaa ctc tgc ttc agt gca aag ttc aga cct act aag caa aac      2121
Ile Leu Lys Leu Cys Phe Ser Ala Lys Phe Arg Pro Thr Lys Gln Asn
                680                 685                 690

aat caa gtg gcc att gta tat aac atc aca ctt gat gca gat gga ttt      2169
Asn Gln Val Ala Ile Val Tyr Asn Ile Thr Leu Asp Ala Asp Gly Phe
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tca tcc aga gta acc tcc agg ggg tta ttt aaa gaa aac aat gaa agg      2217
Ser Ser Arg Val Thr Ser Arg Gly Leu Phe Lys Glu Asn Asn Glu Arg
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tgc ctg cag aag aat atg gta gta aat caa gca cag agt tgc ccc gag      2265
Cys Leu Gln Lys Asn Met Val Val Asn Gln Ala Gln Ser Cys Pro Glu
    725                 730                 735

cac atc att tat ata cag gag ccc tct gat gtt gtc aac tct ttg gat      2313
His Ile Ile Tyr Ile Gln Glu Pro Ser Asp Val Val Asn Ser Leu Asp
740                 745                 750                 755

ttg cgt gtg gac atc agt ctg gaa aac cct ggc act agc cct gcc ctt      2361
Leu Arg Val Asp Ile Ser Leu Glu Asn Pro Gly Thr Ser Pro Ala Leu
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gaa gcc tat tct gag act gcc aag gtc ttc agt att cct ttc cac aaa      2409
Glu Ala Tyr Ser Glu Thr Ala Lys Val Phe Ser Ile Pro Phe His Lys
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gac tgt ggt gag gat gga ctt tgc att tct gat cta gtc cta gat gtc      2457
Asp Cys Gly Glu Asp Gly Leu Cys Ile Ser Asp Leu Val Leu Asp Val
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cga caa ata cca gct gct caa gaa caa ccc ttt att gtc agc aac caa      2505
Arg Gln Ile Pro Ala Ala Gln Glu Gln Pro Phe Ile Val Ser Asn Gln
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aac aaa agg tta aca ttt tca gta aca ctg aaa aat aaa agg gaa agt      2553
Asn Lys Arg Leu Thr Phe Ser Val Thr Leu Lys Asn Lys Arg Glu Ser
820                 825                 830                 835

gca tac aac act gga att gtt gtt gat ttt tca gaa aac ttg ttt ttt      2601
Ala Tyr Asn Thr Gly Ile Val Val Asp Phe Ser Glu Asn Leu Phe Phe
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gca tca ttc tcc cta ccg gtt gat ggg aca gaa gta aca tgc cag gtg      2649
Ala Ser Phe Ser Leu Pro Val Asp Gly Thr Glu Val Thr Cys Gln Val
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gct gca tct cag aag tct gtt gcc tgc gat gta ggc tac cct gct tta      2697
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caa aac ctt cag aat cag gcg tct ctc agt ttc caa gcc tta agt gaa      2793
Gln Asn Leu Gln Asn Gln Ala Ser Leu Ser Phe Gln Ala Leu Ser Glu
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agc caa gaa gaa aac aag gct gat aat ttg gtc aac ctc aaa att cct      2841
Ser Gln Glu Glu Asn Lys Ala Asp Asn Leu Val Asn Leu Lys Ile Pro
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ctc ctg tat gat gct gaa att cac tta aca aga tct acc aac ata aat      2889
Leu Leu Tyr Asp Ala Glu Ile His Leu Thr Arg Ser Thr Asn Ile Asn
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ttt tat gaa atc tct tcg gat ggg aat gtt cct tca atc gtg cac agt      2937
Phe Tyr Glu Ile Ser Ser Asp Gly Asn Val Pro Ser Ile Val His Ser
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Gly Ser Val Pro Val Ser Met Ala Thr Val Ile Ile His Ile Pro Gln
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Thr Asp Lys Ala Gly  Asp Ile Ser Cys Asn  Ala Asp Ile Asn Pro
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ctg aaa ata gga caa  aca tct tct tct gta  tct ttc aaa agt gaa       3168
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Ser Asn Val Thr Cys  Trp Leu Lys Asp Val  His Met Lys Gly Glu
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Tyr Phe Val Asn Val  Thr Thr Arg Ile Trp  Asn Gly Thr Phe Ala
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Arg Lys Tyr Glu Lys  Met Thr Lys Asn Pro  Asp Glu Ile Asp Glu
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acc aca gag ctc agt  agc tgaaccagca gacctacctg cagtgggaac          3621
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His Ser Ser Tyr Leu Gly Tyr Ser Val Ala Ala Ile Ser Thr Gly Glu
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Ser Thr His Phe Val Ala Gly Ala Pro Arg Ala Asn Tyr Thr Gly Gln
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Ala Pro Met Tyr Met Ser Asp Leu Lys Lys Glu Glu Gly Arg Val Tyr
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Leu Phe Thr Ile Lys Lys Gly Ile Leu Gly Gln His Gln Phe Leu Glu
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Gly Pro Glu Gly Ile Glu Asn Thr Arg Phe Gly Ser Ala Ile Ala Ala
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Leu Ser Asp Ile Asn Met Asp Gly Phe Asn Asp Val Ile Val Gly Ser
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Pro Leu Glu Asn Gln Asn Ser Gly Ala Val Tyr Ile Tyr Asn Gly His
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Gln Gly Thr Ile Arg Thr Lys Tyr Ser Gln Lys Ile Leu Gly Ser Asp
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Gly Ala Phe Arg Ser His Leu Gln Tyr Phe Gly Arg Ser Leu Asp Gly
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Tyr Gly Asp Leu Asn Gly Asp Ser Ile Thr Asp Val Ser Ile Gly Ala
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Phe Gly Gln Val Val Gln Leu Trp Ser Gln Ser Ile Ala Asp Val Ala
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Ile Glu Ala Ser Phe Thr Pro Glu Lys Ile Thr Leu Val Asn Lys Asn
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Ala Gln Ile Ile Leu Lys Leu Cys Phe Ser Ala Lys Phe Arg Pro Thr
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Lys Gln Asn Asn Gln Val Ala Ile Val Tyr Asn Ile Thr Leu Asp Ala
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Asp Gly Phe Ser Ser Arg Val Thr Ser Arg Gly Leu Phe Lys Glu Asn
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Asn Glu Arg Cys Leu Gln Lys Asn Met Val Val Asn Gln Ala Gln Ser
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Cys Pro Glu His Ile Ile Tyr Ile Gln Glu Pro Ser Asp Val Val Asn
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Ser Leu Asp Leu Arg Val Asp Ile Ser Leu Glu Asn Pro Gly Thr Ser
755 760 765

Pro Ala Leu Glu Ala Tyr Ser Glu Thr Ala Lys Val Phe Ser Ile Pro
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Phe His Lys Asp Cys Gly Glu Asp Gly Leu Cys Ile Ser Asp Leu Val
785 790 795 800

Leu Asp Val Arg Gln Ile Pro Ala Ala Gln Glu Gln Pro Phe Ile Val
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Ser Asn Gln Asn Lys Arg Leu Thr Phe Ser Val Thr Leu Lys Asn Lys
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Arg Glu Ser Ala Tyr Asn Thr Gly Ile Val Val Asp Phe Ser Glu Asn
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Leu Phe Phe Ala Ser Phe Ser Leu Pro Val Asp Gly Thr Glu Val Thr
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Cys Gln Val Ala Ala Ser Gln Lys Ser Val Ala Cys Asp Val Gly Tyr
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Pro Ala Leu Lys Arg Glu Gln Gln Val Thr Phe Thr Ile Asn Phe Asp
885 890 895

Phe Asn Leu Gln Asn Leu Gln Asn Gln Ala Ser Leu Ser Phe Gln Ala
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Leu Ser Glu Ser Gln Glu Glu Asn Lys Ala Asp Asn Leu Val Asn Leu
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Lys Ile Pro Leu Leu Tyr Asp Ala Glu Ile His Leu Thr Arg Ser Thr
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Asn Ile Asn Phe Tyr Glu Ile Ser Ser Asp Gly Asn Val Pro Ser Ile
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Val His Ser Phe Glu Asp Val Gly Pro Lys Phe Ile Phe Ser Leu Lys
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Val Thr Thr Gly Ser Val Pro Val Ser Met Ala Thr Val Ile Ile His
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Ile Pro Gln Tyr Thr Lys Glu Lys Asn Pro Leu Met Tyr Leu Thr Gly
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Val Gln Thr Asp Lys Ala Gly Asp Ile Ser Cys Asn Ala Asp Ile
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Asn Pro Leu Lys Ile Gly Gln Thr Ser Ser Ser Val Ser Phe Lys
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Ser Glu Asn Phe Arg His Thr Lys Glu Leu Asn Cys Arg Thr Ala
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Ser Cys Ser Asn Val Thr Cys Trp Leu Lys Asp Val His Met Lys
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Phe Ala Ser Ser Thr Phe Gln Thr Val Gln Leu Thr Ala Ala Ala
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Glu Ile Asn Thr Tyr Asn Pro Glu Ile Tyr Val Ile Glu Asp Asn
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Thr Val Thr Ile Pro Leu Met Ile Met Lys Pro Asp Glu Lys Ala
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Glu Val Pro Thr Gly Val Ile Ile Gly Ser Ile Ile Ala Gly Ile
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Leu Leu Leu Leu Ala Leu Val Ala Ile Leu Trp Lys Leu Gly Phe
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